Aging & Rehabilitation

An Interdisciplinary Research Seminar Series

UNIVERSITY OF FLORIDA

Sponsors

Department of Veteran Affairs

- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)

UF College of Medicine

- Institute on Aging
- Department of Aging and Geriatric Research

UF College of Public Health and Health Professions

- Brooks Center for Rehabilitation Studies

UF College of Liberal Arts and Sciences

- Center for Gerontological Studies

UF McKnight Brain Institute

UF College of Nursing

Schedule

- August 29th, 2005 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- HPNP Room – G103

CYBER SEMINAR VENUES

- VA RORC, Conference Room, Suite 350
- VA BRRC, VA Nursing Home, Room 271-12
- UF Brooks Center Conference Room, Jacksonville

Themes

- Basic Science (C. Leeuwenburgh)
- Clinical Science (R. Beyth)
- Outcomes / Health Policy (E. Andresen)
- Behavioral and Social Research (M. Marsiske)
- Cutting Edge / New Research (T. Foster/ J. Aris)

“Alzheimer-type amyloidosis and cognition in transgenic mouse models”

David R. Borchelt, Professor of Neuroscience

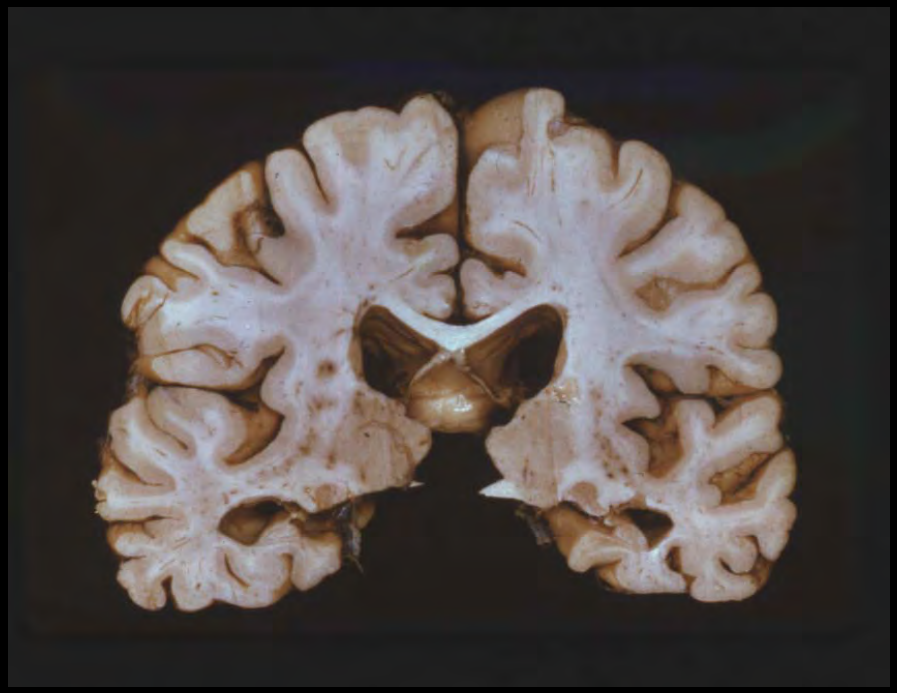
Guilian Xu and Joanna Jankowsky – Transgenic Mouse Models of Alzheimer-Pathology

Tatiana Melinkova and Alena Savonenko – Assessing Cognition in Mouse Models of AD

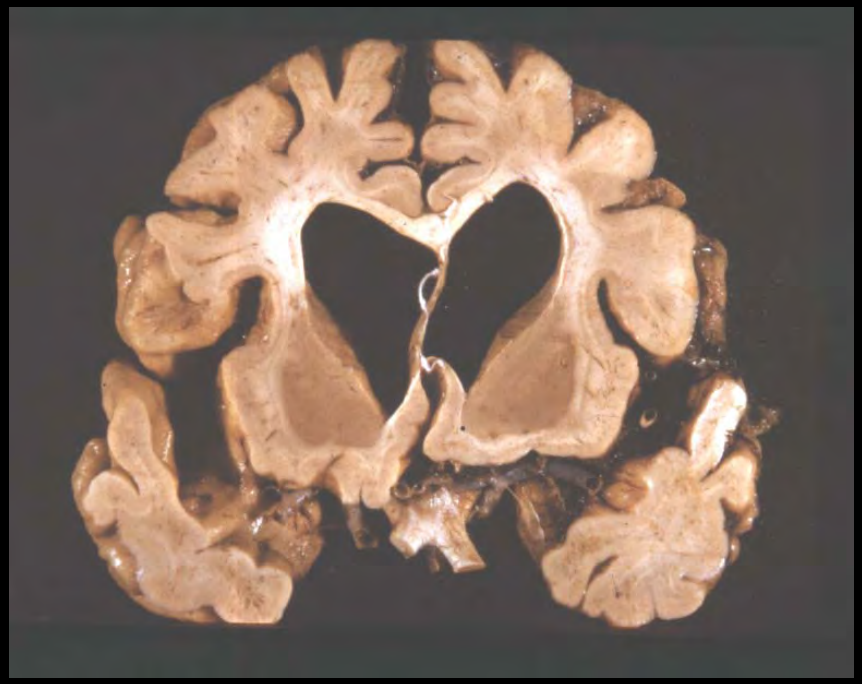
Alzheimer's Disease

- Loss of memory
- Impaired cognition
- Problems with activities of daily living
- Changes in personality

Moderate Atrophy



Severe Atrophy

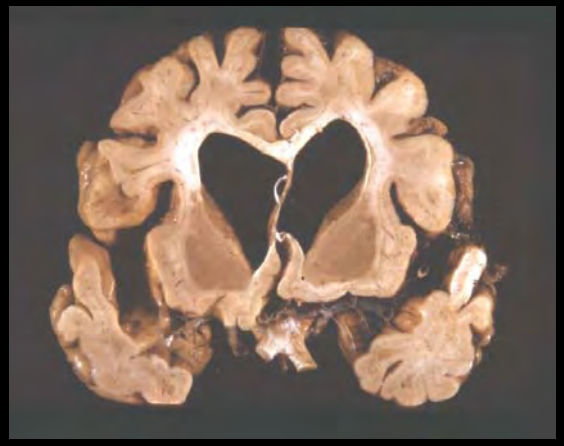


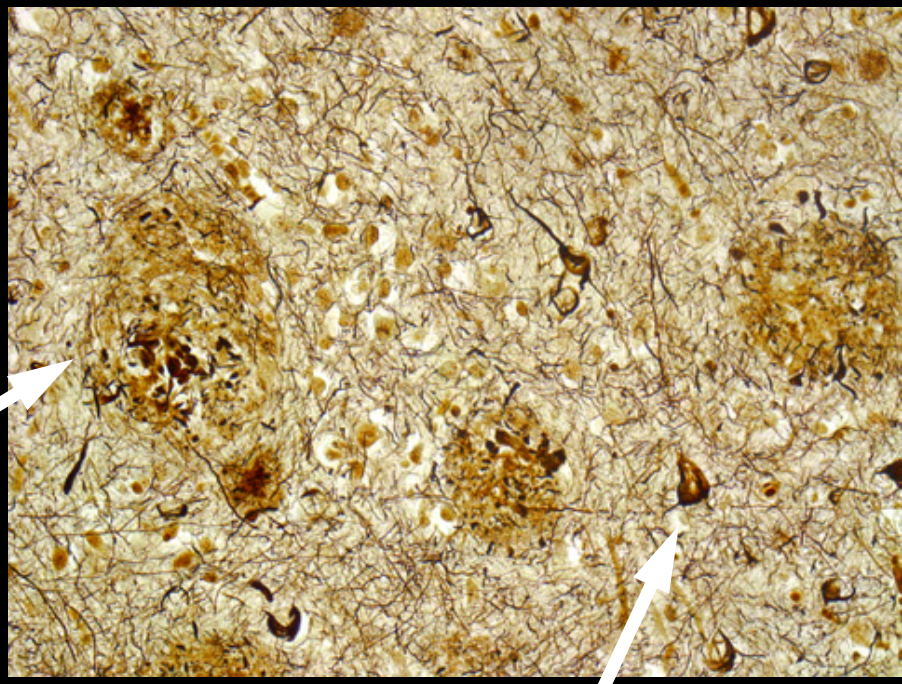
Neurochemical Deficits in AD

- Cholinergic system
  - ChAT ↓
  - AChE ↓
  - Muscarinic receptor ↓
  - Nicotinic receptor ↓
- Neuropeptides
  - Somatostatin ↓
  - Somatostatin receptor ↓

Aricept targets AChE to help raise the levels of acetylcholine.

Disease-Specific Lesions in Alzheimer's Disease



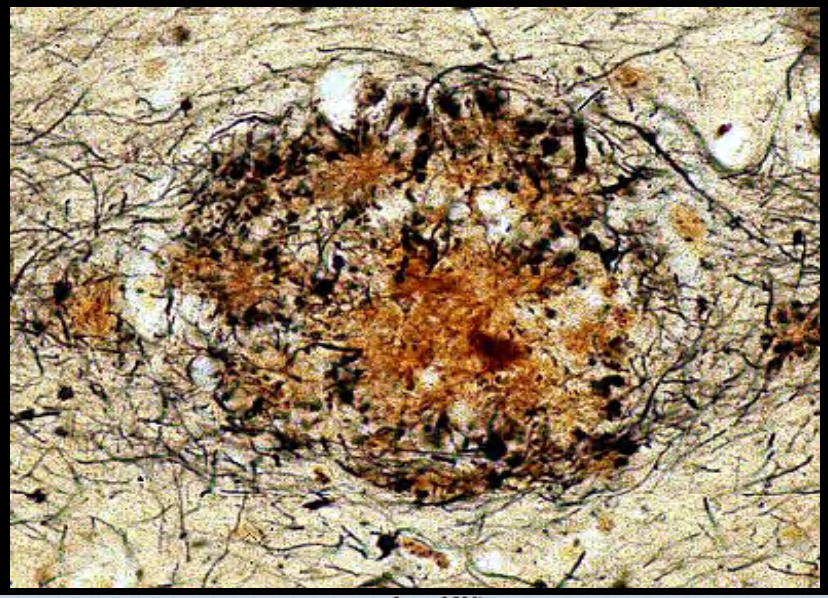


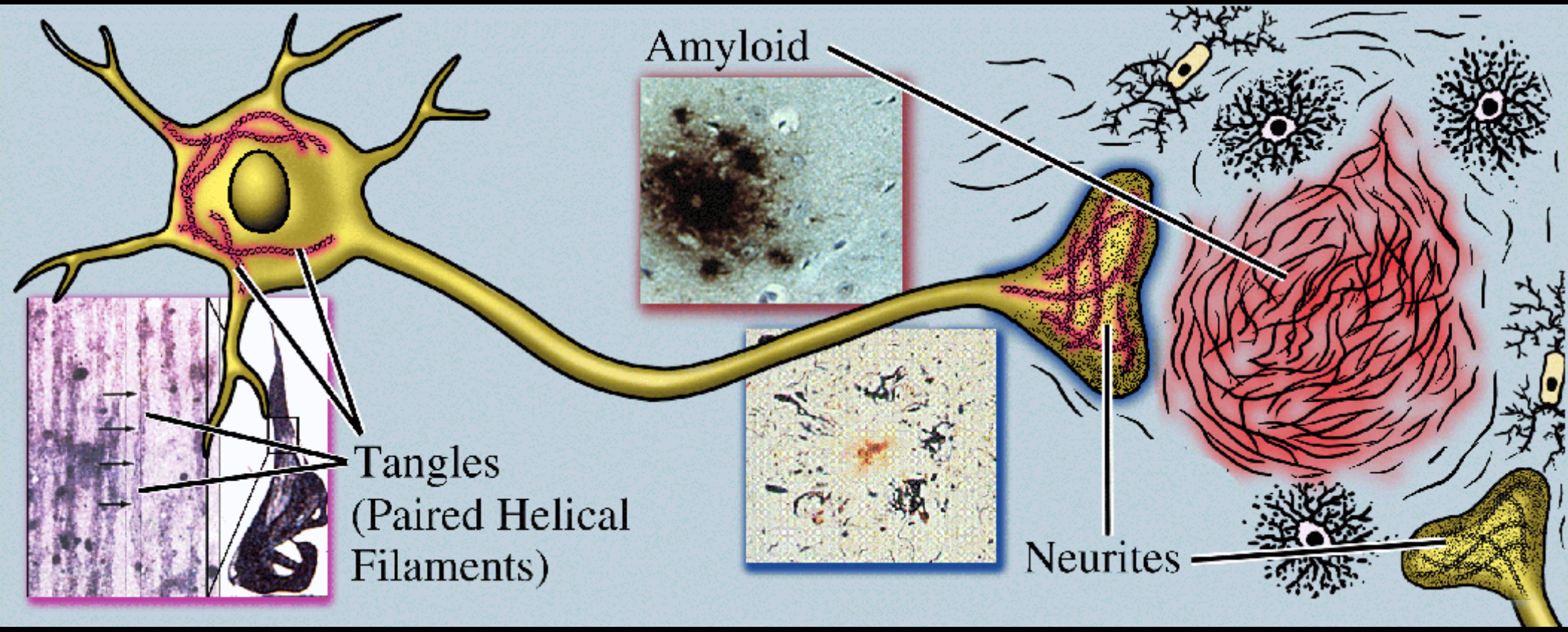
The brains of patients with Alzheimer's disease usually contain 100's to 1000's of lesions per tissue section

Alzheimer's Disease Pathology

Amyloid plaques are formed when the β-amyloid peptide abnormally aggregates in the fluid space of the brain.

Neurofibrillary tangles form when a protein call tau abnormally aggregates in the cytoplasm of CNS neurons.



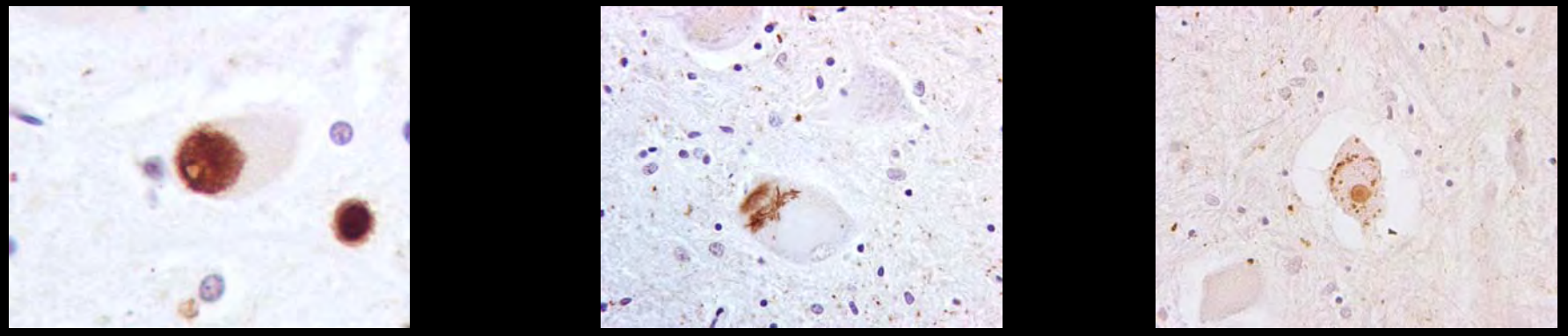


Protein Aggregation in Familial and Sporadic Neurodegenerative Diseases

Disease	Pathology	Protein Culprit	Transgenic mouse model
Alzheimer's	Extracellular amyloid cytoplasmic tangles	Aβ, Tau	Mutant APP, APP:PS1 or mutant tau
ALS	Cytoplasmic inclusions	SOD1 Only in SOD1-linked fALS	Mutant SOD1
Huntington's	Intranuclear and cytoplasmic inclusions	Mutant huntingtin	Full-length and N-terminal fragments of huntingtin with expanded repeats
CJD & mad cow Disease	Kuru plaques	Prion proteins	Wild-type and Mutant prion protein
Parkinson's	Lewy bodies	α-synuclein	Mutant α-synuclein

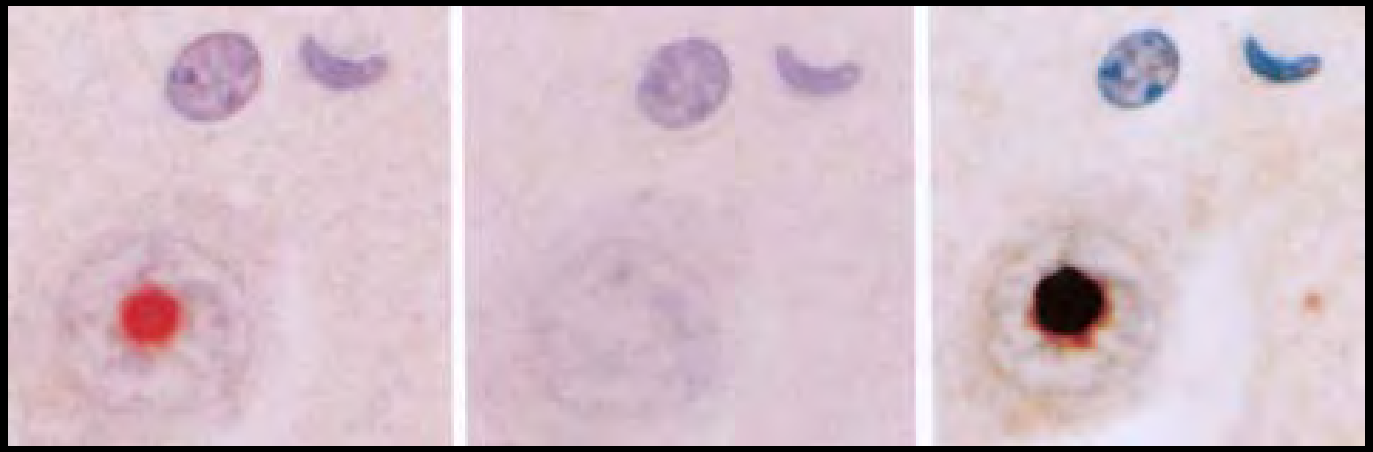
Protein Aggregates in ALS

Disease	Aggregate	Component	Transgenic mice model
Familial ALS	Cytoplasmic inclusions	SOD1	Mutant SOD1
Sporadic ALS	Cytoplasmic inclusions	Ubiquitin, neurofilament, Peripherin, Cystatin C	Mutant & wt NFs Wt-peripherin

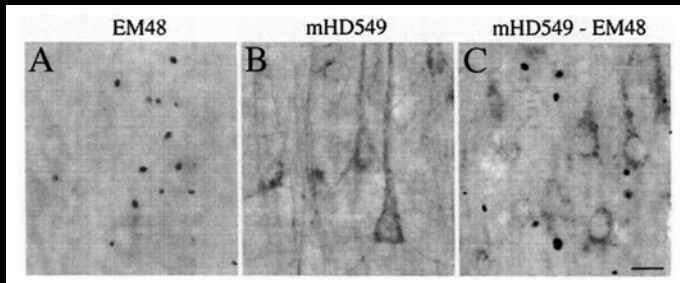


Ubiquitin-immunoreactive inclusions in sporadic ALS

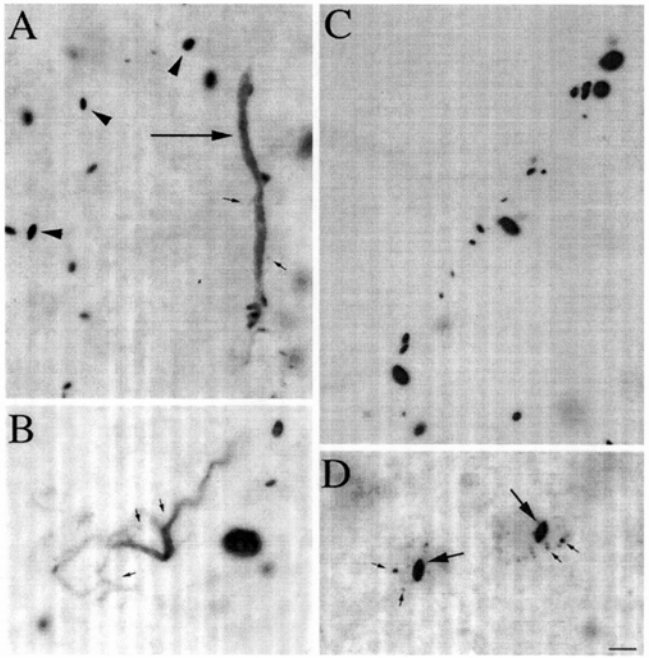
A Ubiquitinated Form of Mutant Huntingtin Aggregates and Accumulates in the Nucleus



Anti huntingtin | Destain | Anti ubiquitin



Mutant Huntingtin Aggregates Accumulate in the Cytoplasm



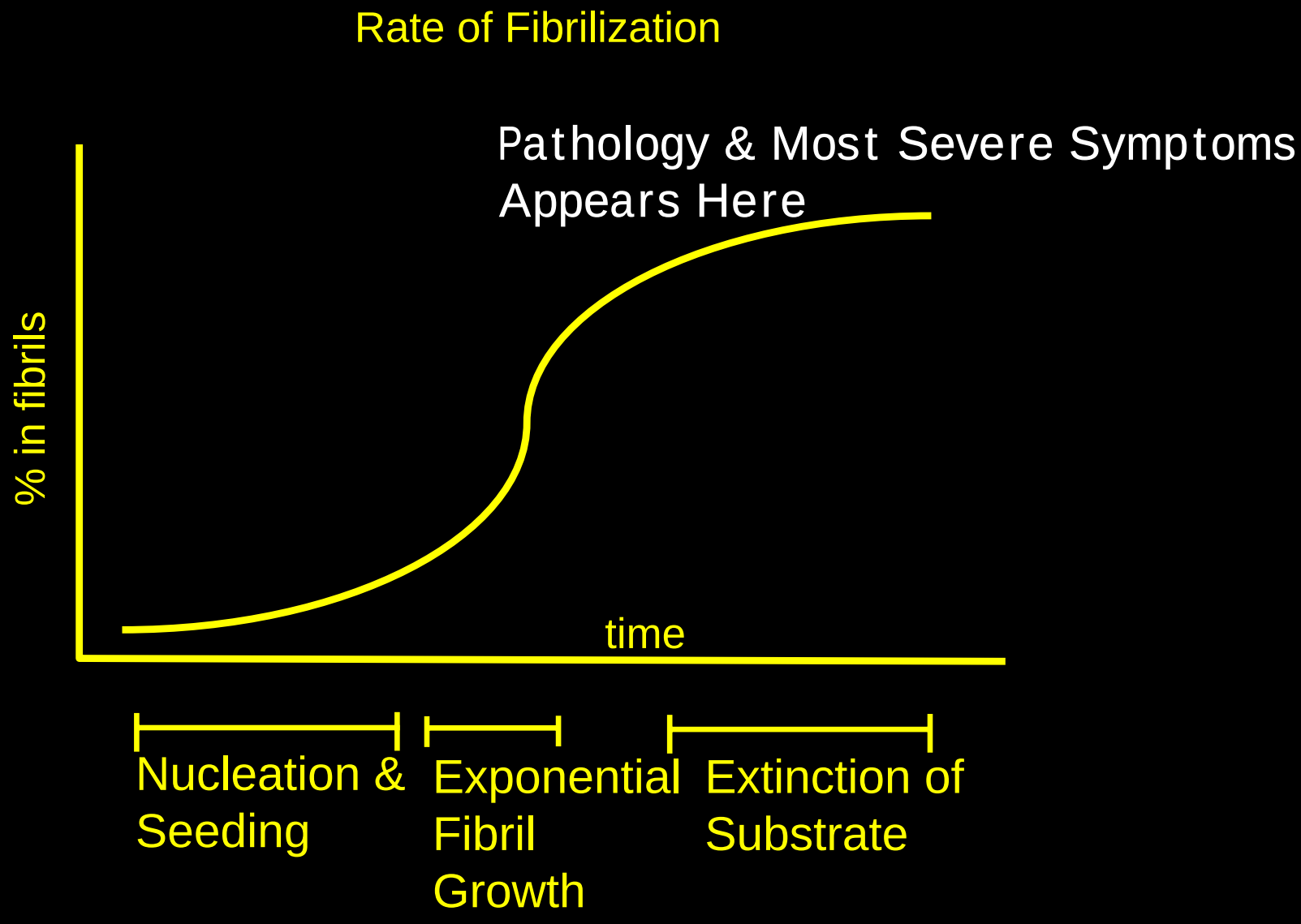
The two most important risk factors for neuro-degenerative disease are genetics and age.

With sporadic forms of disease, such as Alzheimer's disease or ALS, age is the most prominent risk factor.

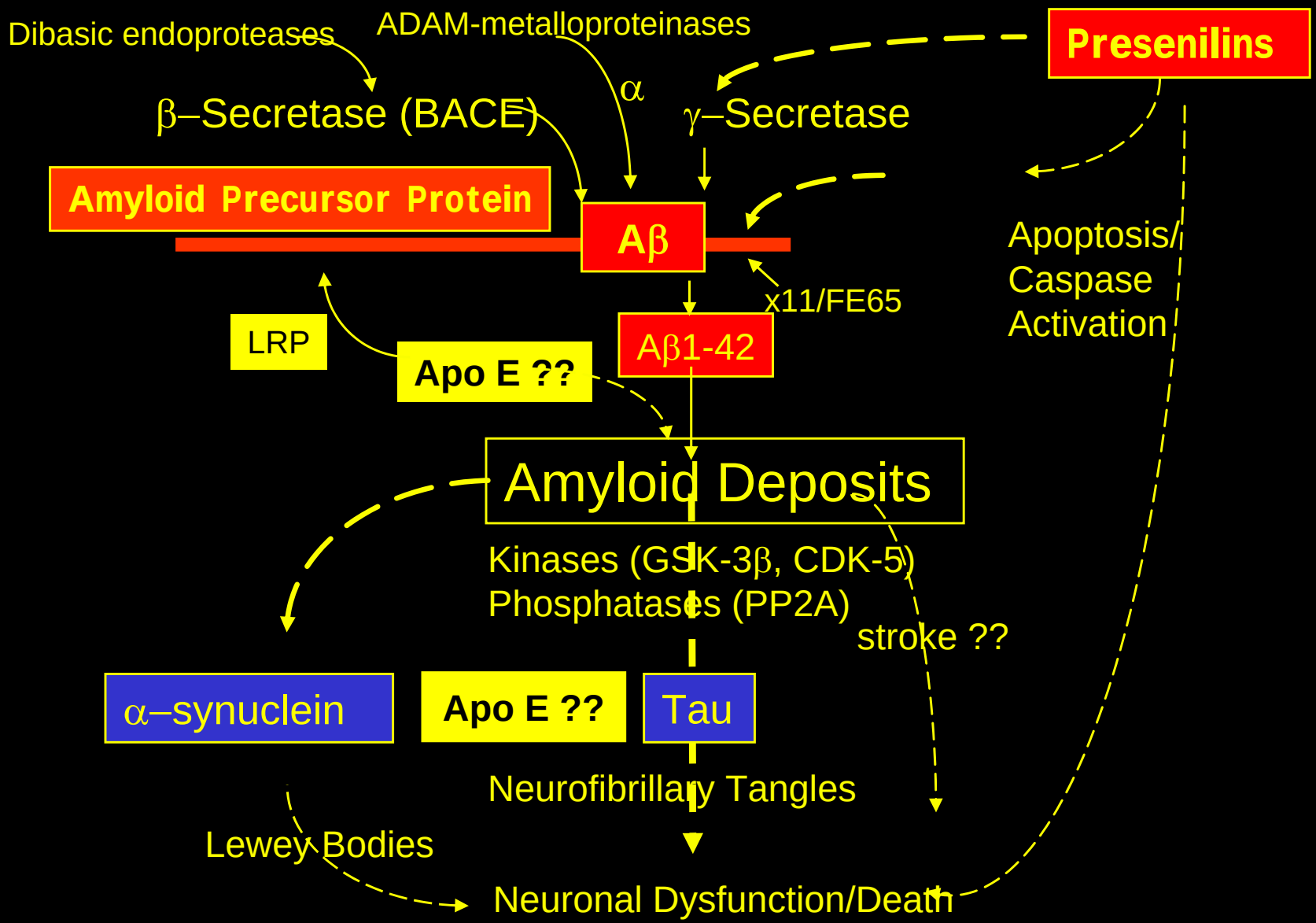
In familial forms, the obvious risk factor is genetics, but age also plays a role.

Why??

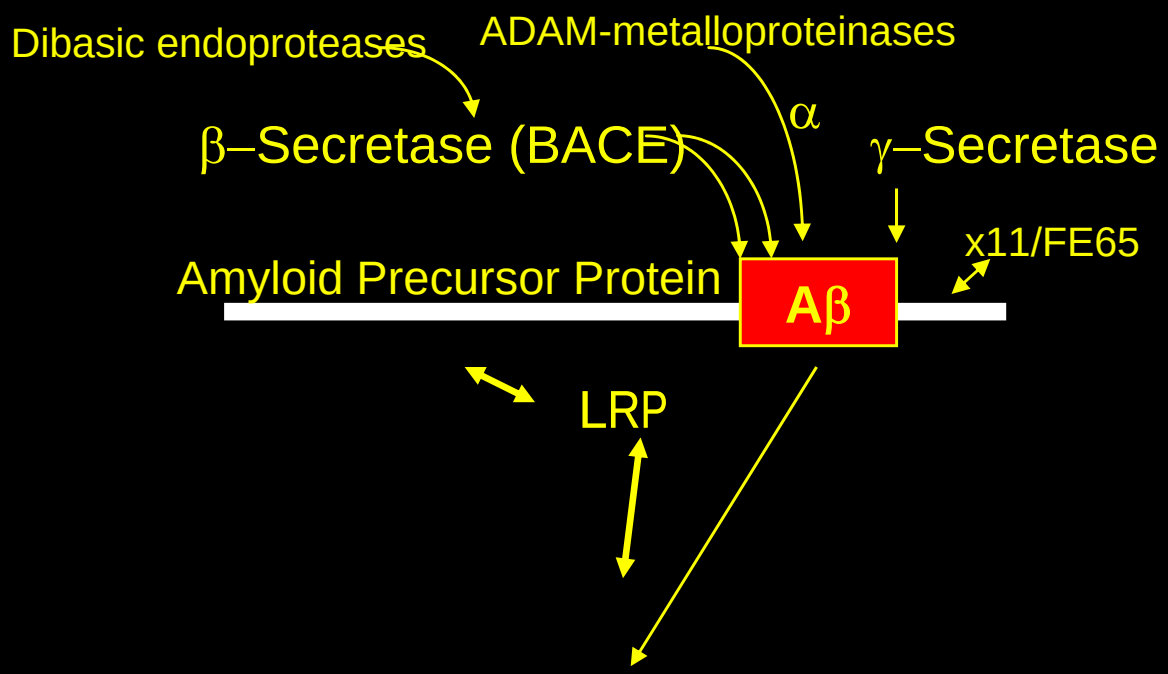
Kinetics of Protein Aggregation *In Vitro*



Genetic Dissection of Pathogenic Pathways in Alzheimer's Disease



The Amyloid Pathway



Aβ11-40

Aβ1-40

Aβ17-40

Aβ11-42

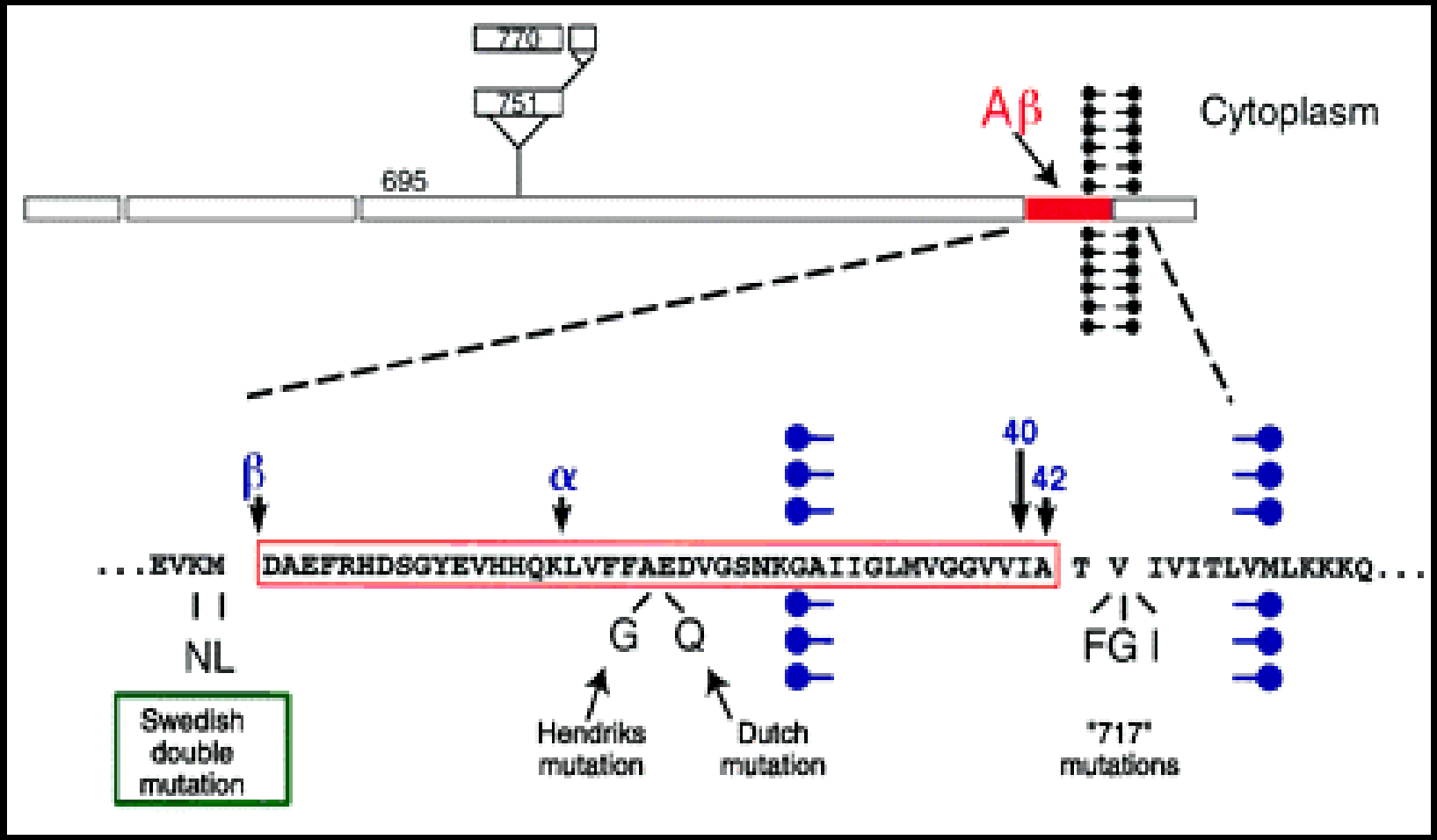
Aβ17-42

Aβ1-42

Rapidly aggregates in vitro

+ Peptides terminating at residue 38, 39, and 43

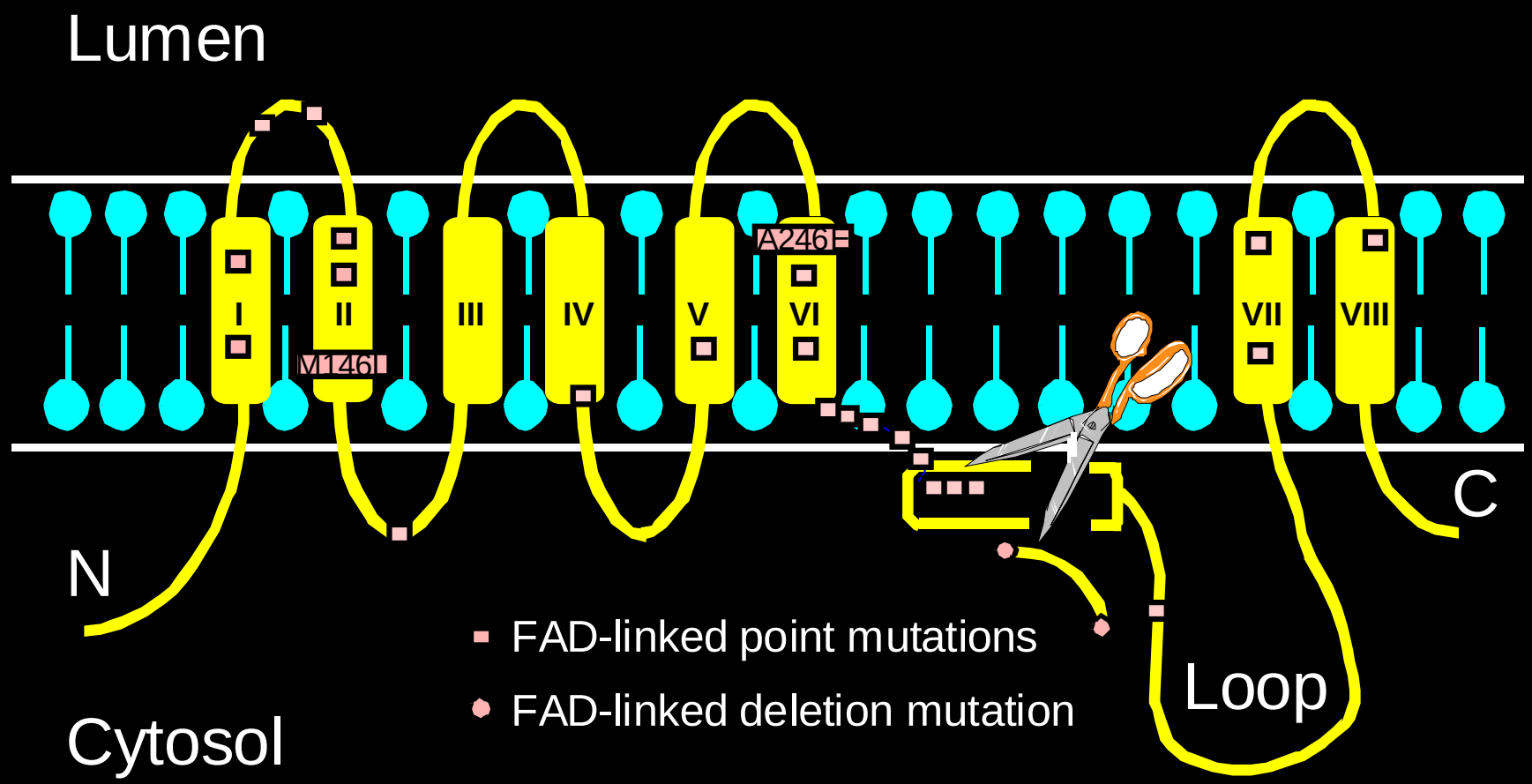
Mutations in the Amyloid Precursor Protein Alter Endoproteolytic Processing



The net effect of these mutations is to raise the amount of Aβ42 produced per precursor peptide processed.

Review by Price et al., Science 282:1079-83 (1998)

PS1: Topology, Cleavage Site, and Mutations

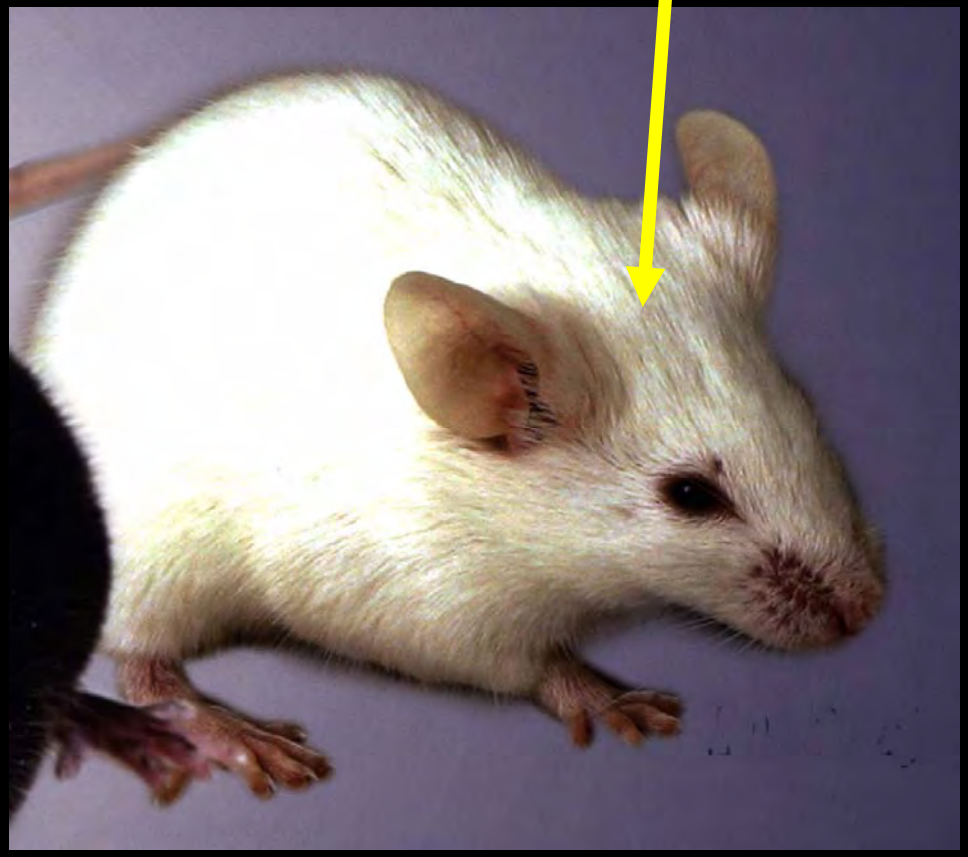


Doan et al., 1996
Thinakaran et al., 1996

Verify amyloid hypothesis through molecular genetics

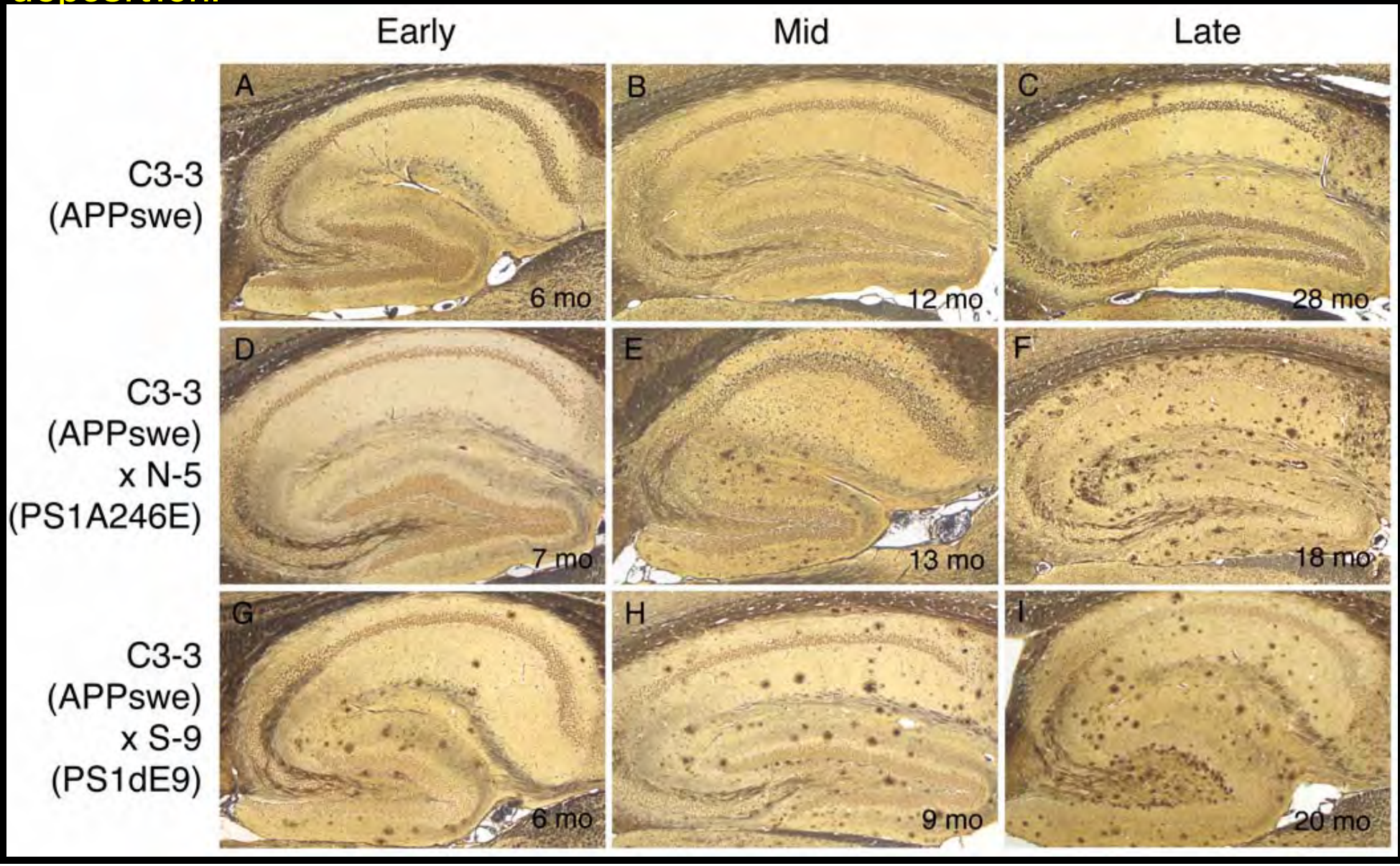
Delete normal mouse genes

Add normal and mutant human genes

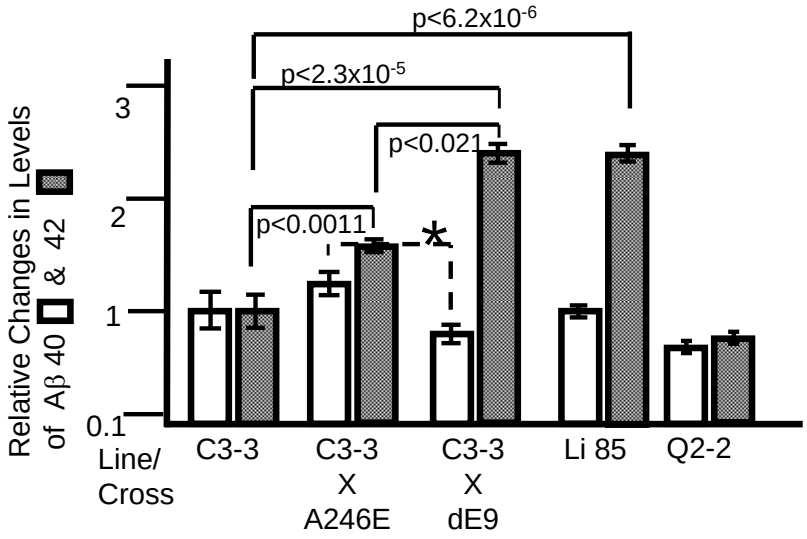


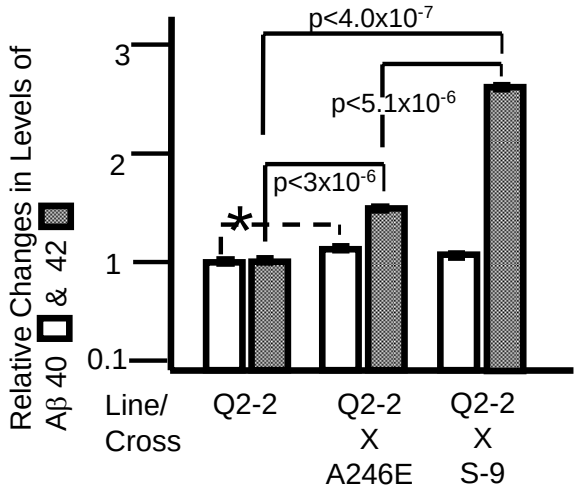
Mice expressing mutant APP (APPswe) produce human Aβ and develop amyloid plaques at very advanced ages (>24 months).

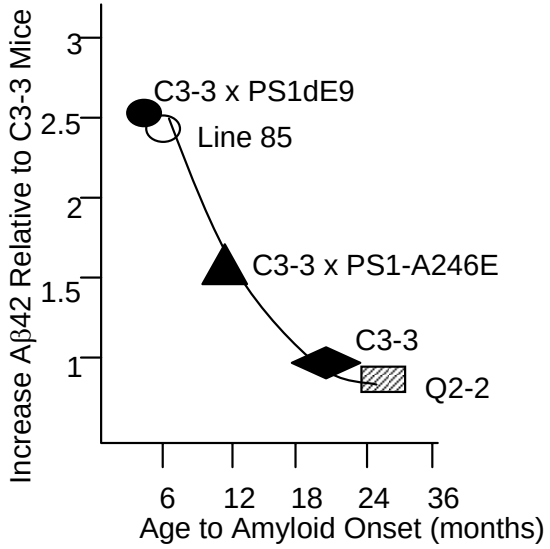
Co-expressing mutant PS1 greatly accelerates the rate of amyloid deposition.

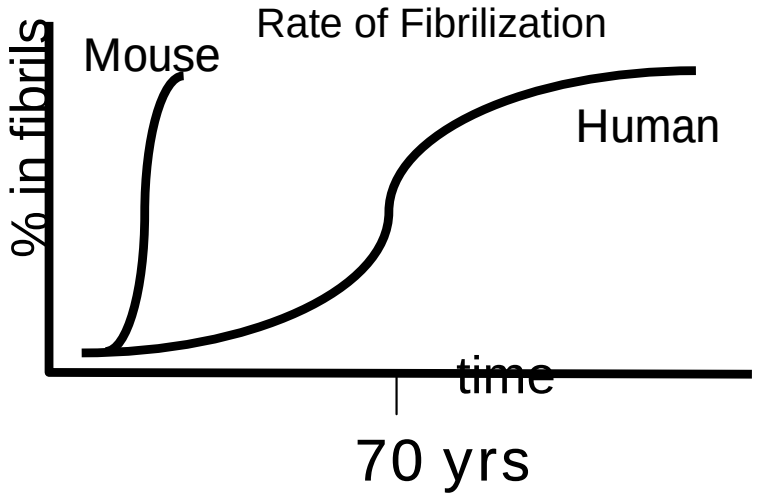


Mutant PS1 Specifically Elevates the Production Of Aβ42 Without Affecting Aβ40; And, the Rate of Amyloid Deposition Is Directly Related to the Relative Production of Aβ42



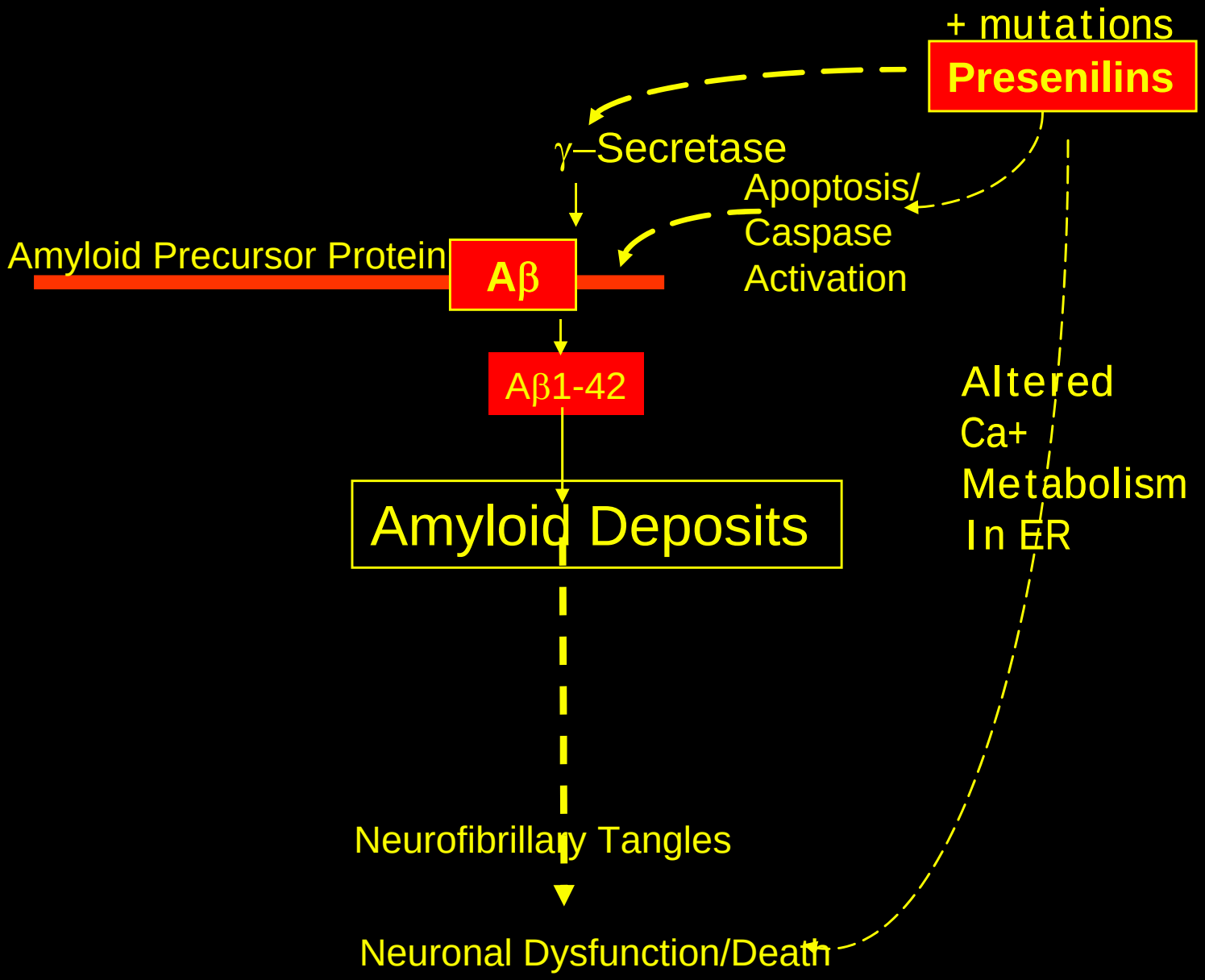






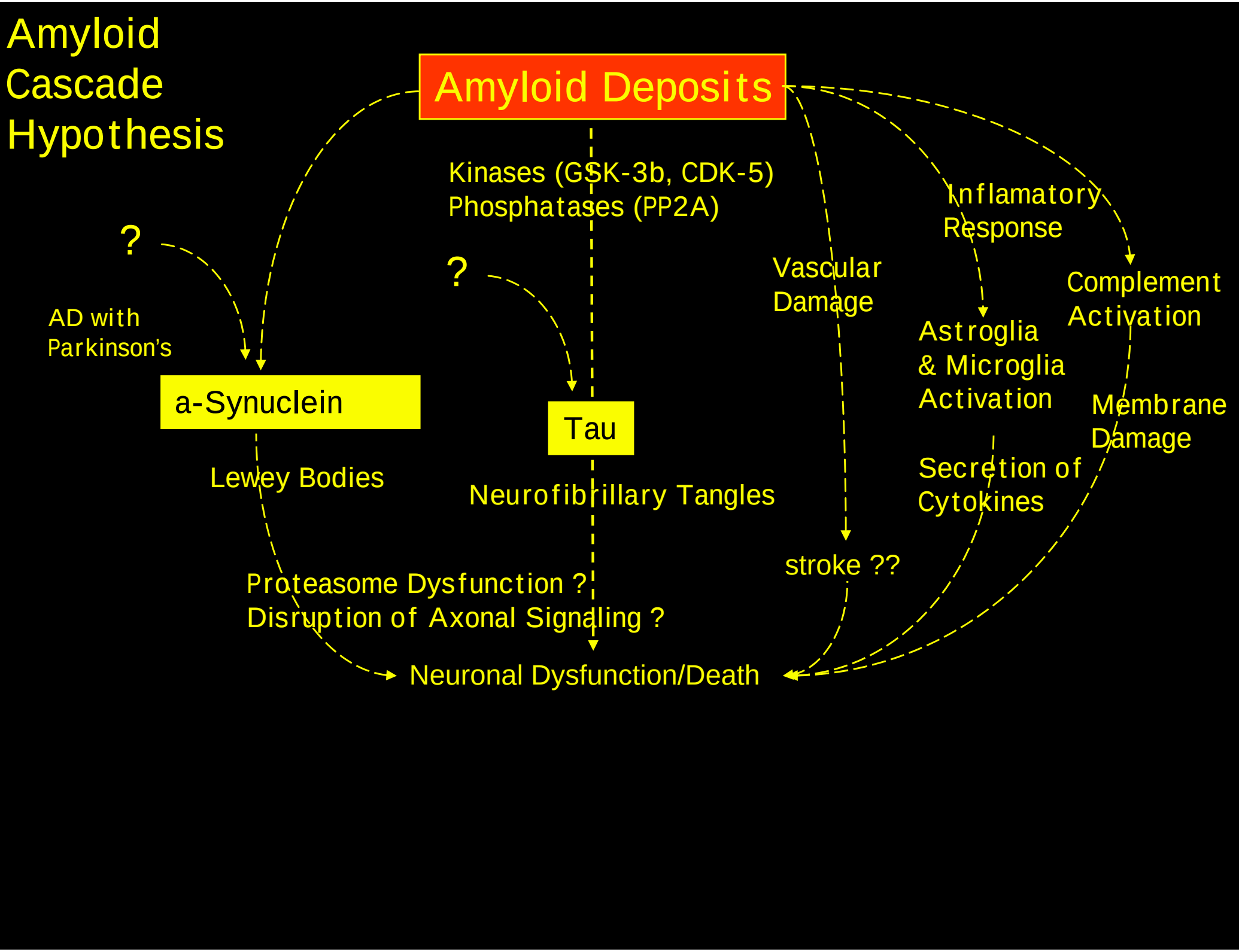
High levels of Aβ42 production are required to produce pathology in the lifespan of mice.

Potential Pathogenic Pathways by which Mutations in Presenilin Cause Alzheimer's Disease

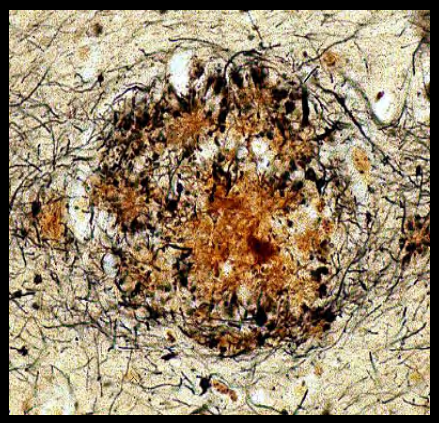


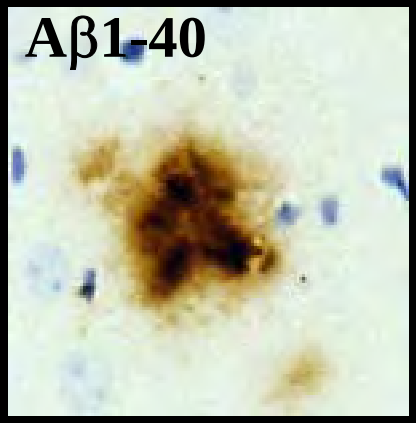
APP
Notch-1
Delta 1
Jagged 2
E-cadherin
Syndecan 3
p75 Neurotrophin recpt
CD44
Erb-B4

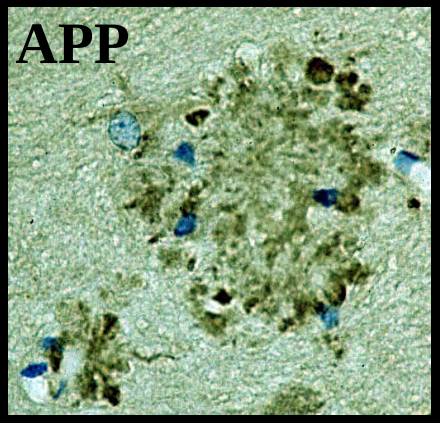
b-catenin
GSK-3b
Filamin
Armadillo-p0071
Plakophilin-related
d-catenin
Sel-10/Cdc4p-rel
Rab 11
Rab GDP-Dissoc Inhib
Bcl-X(L)
Bcl-2
Sorcin
Calmyrin
Calsenilin
mu-Calpain
Calpain
Ubiquilin
HC5/ZETA proteasome

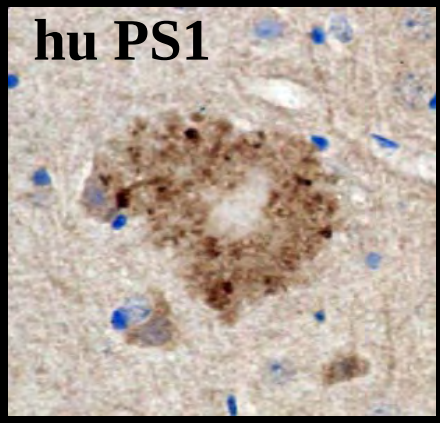

Amyloid Cascade Hypothesis
Amyloid Deposits
Kinases (GSK-3b, CDK-5)
Phosphatases (PP2A)
?
?
AD with Parkinson's
a-Synuclein
Tau
Lewey Bodies
Neurofibrillary Tangles
Vascular Damage
Inflamatory Response
Complement Activation
Astroglia & Microglia Activation
Membrane Damage
Secretion of Cytokines
stroke ??
Proteasome Dysfunction ?
Disruption of Axonal Signaling ?
Neuronal Dysfunction/Death

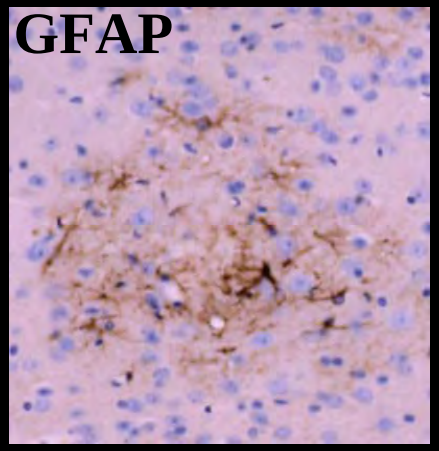
Downstream AD-like pathologies in APPswe/mtPS1 Mice

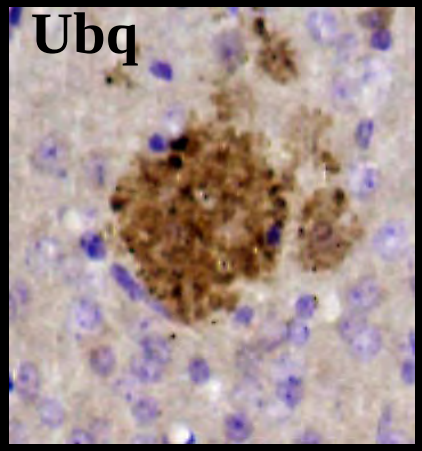


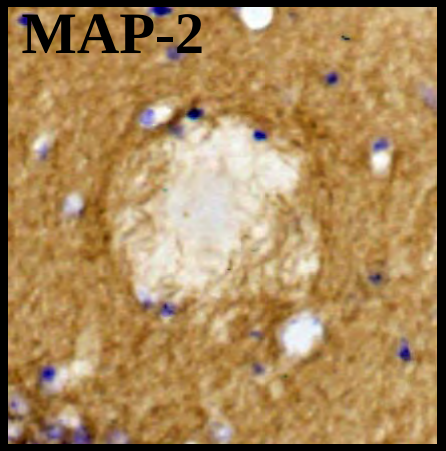


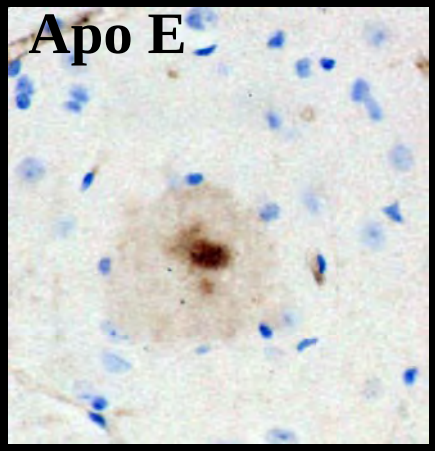










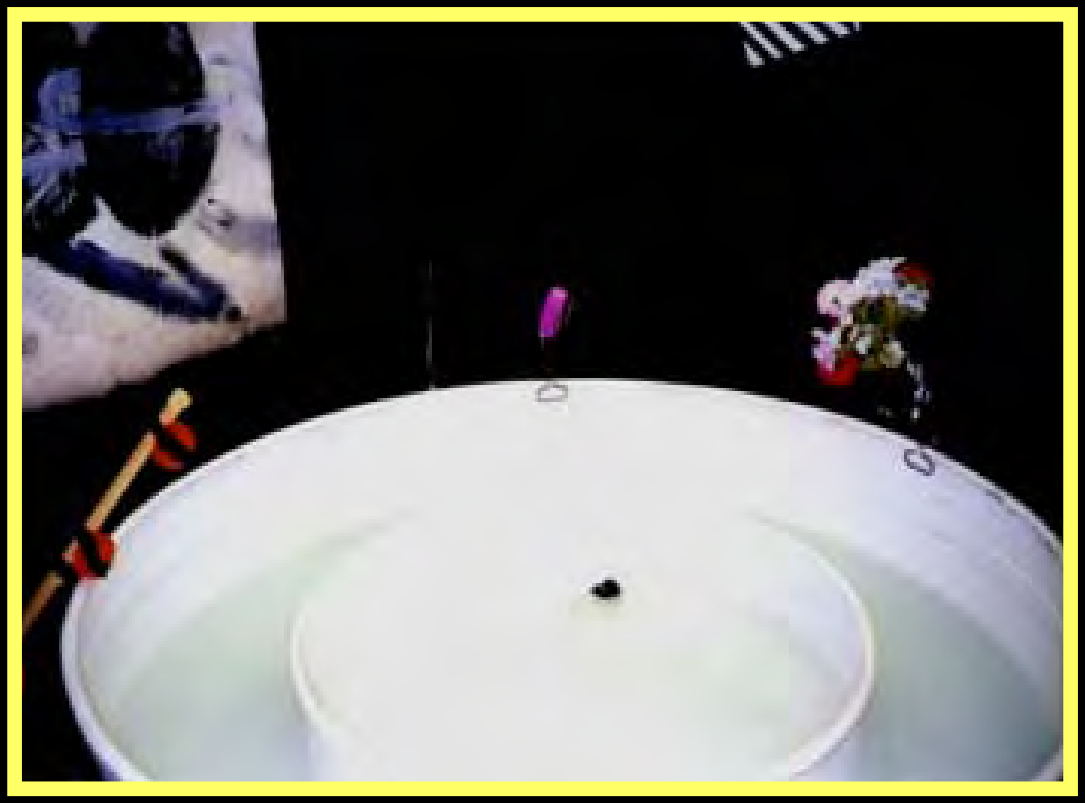


Missing – NFT, microglia activation, complement activation

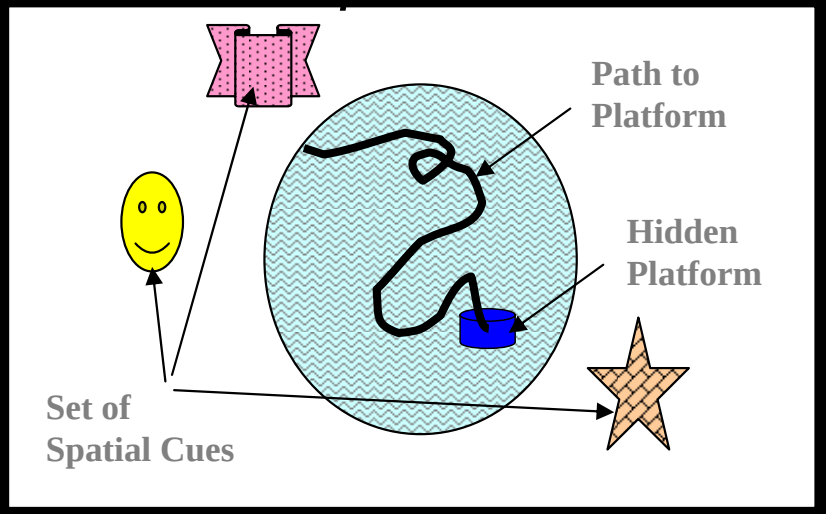
Questions

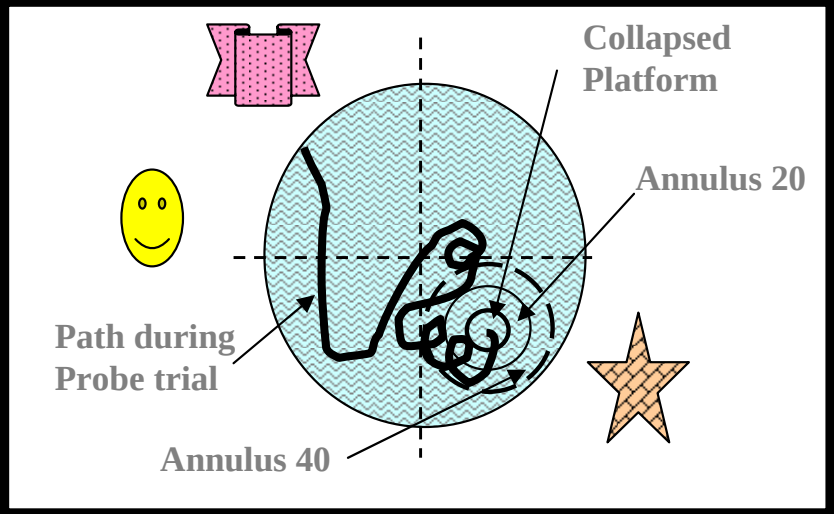
1) Is cognition impaired in the presence of these pathologies?
2) What species of Aβ42 is the toxin?
3) Is Aβ neurotoxic?

Assessing Cognition in Mice



Alena Savonenko, Guilian Xu, and Alicja Markowska



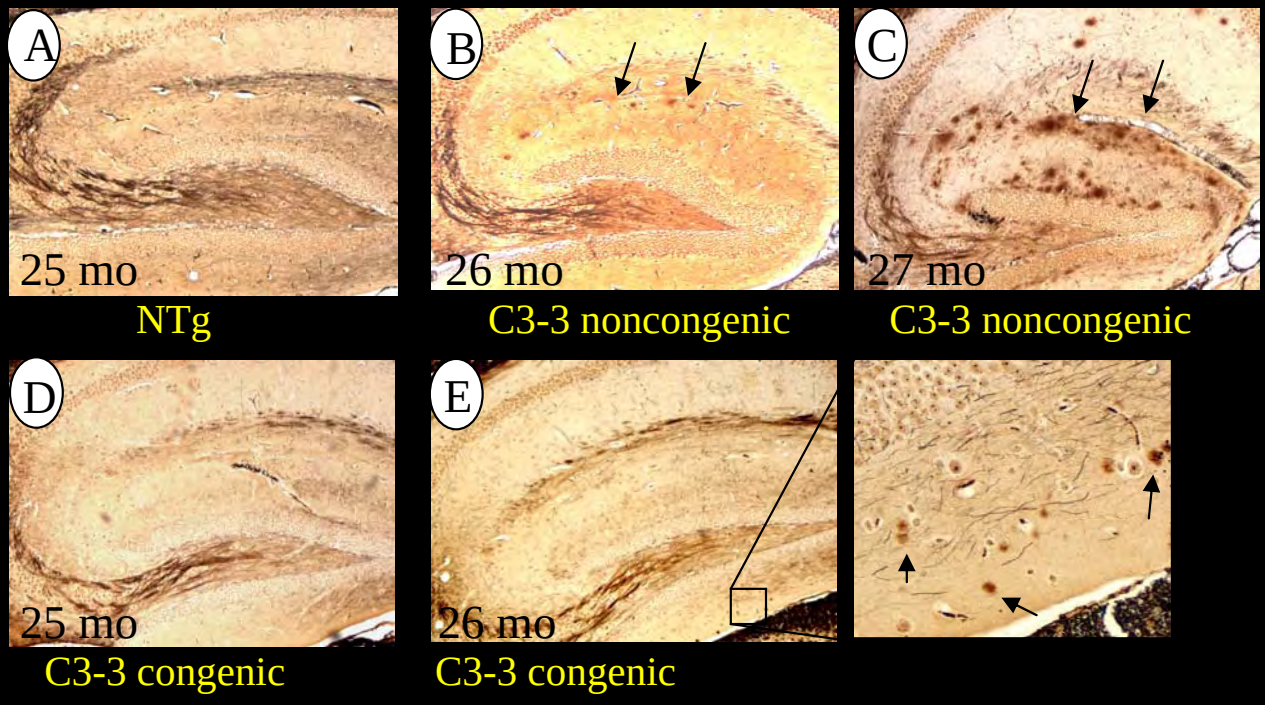


Subjects: Mo/HuAPPswe Congenic (C57BL/6J) Lines C3-3 and E1-2
PS1dE9 Congenic (C57BL/6J) Line S-9

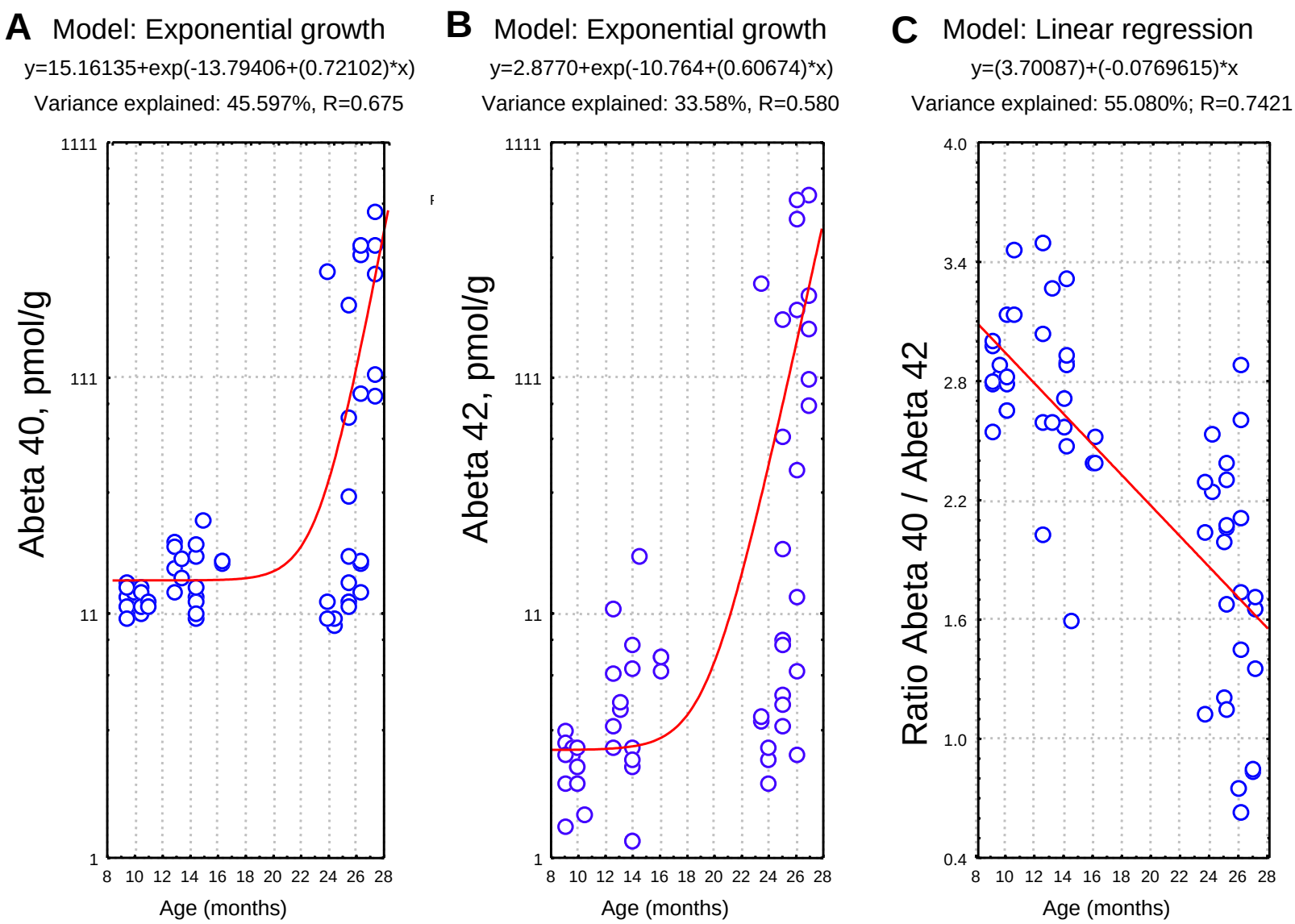
Rationale: C57BL/6J mice perform well in tasks that assess cognitive function. The C3-3 and E1-2 lines of APPswe mice have similar levels of expression and thus allow for control of transgene integrations effects.

Methods: Morris Water Maze, Radial Water Maze, Radial Maze, Y-Maze
Various tasks that assess motor function, vision, and fear.

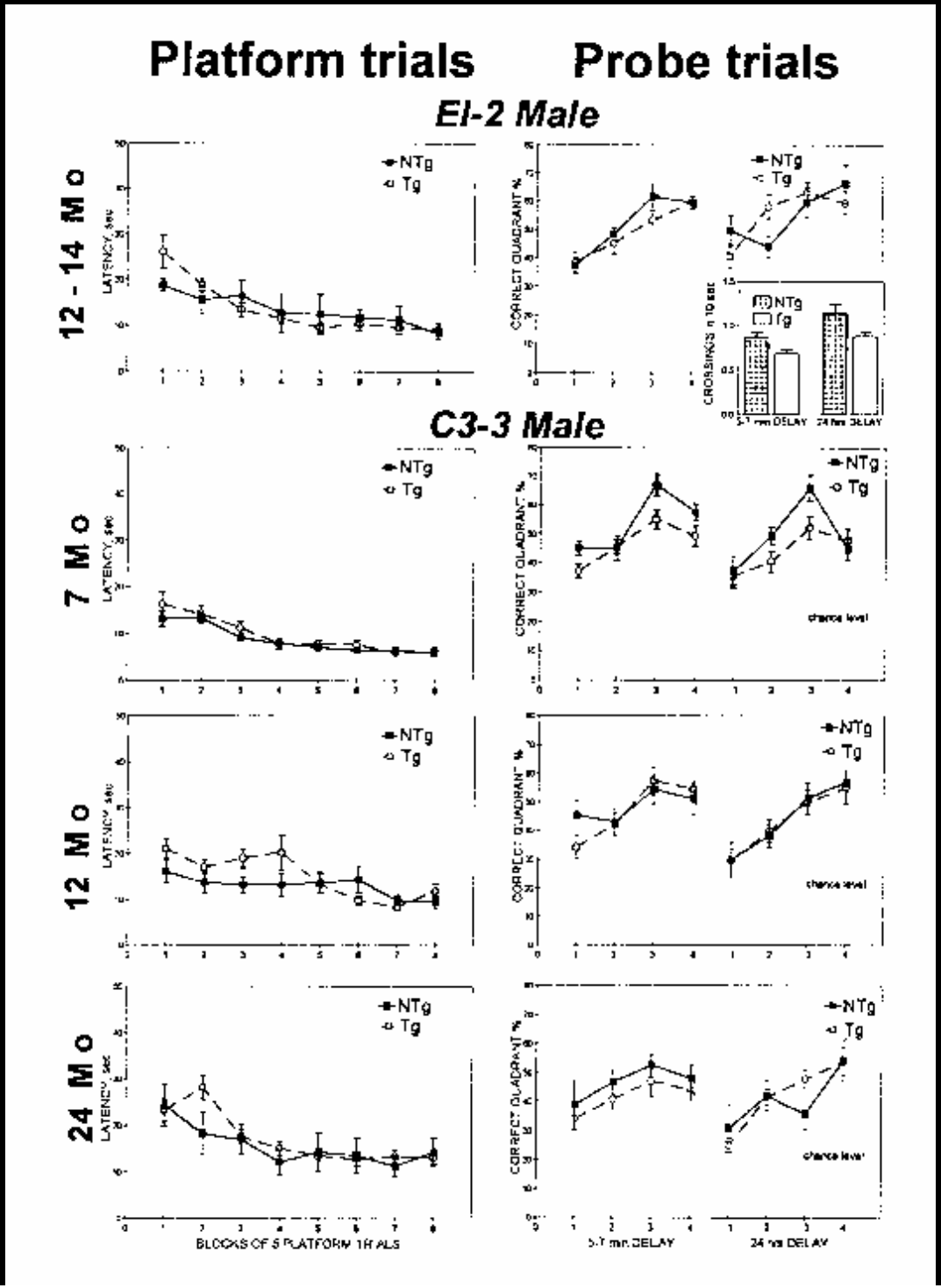
APP Levels and Aβ Deposition in Mo/Hu APPswe Mice



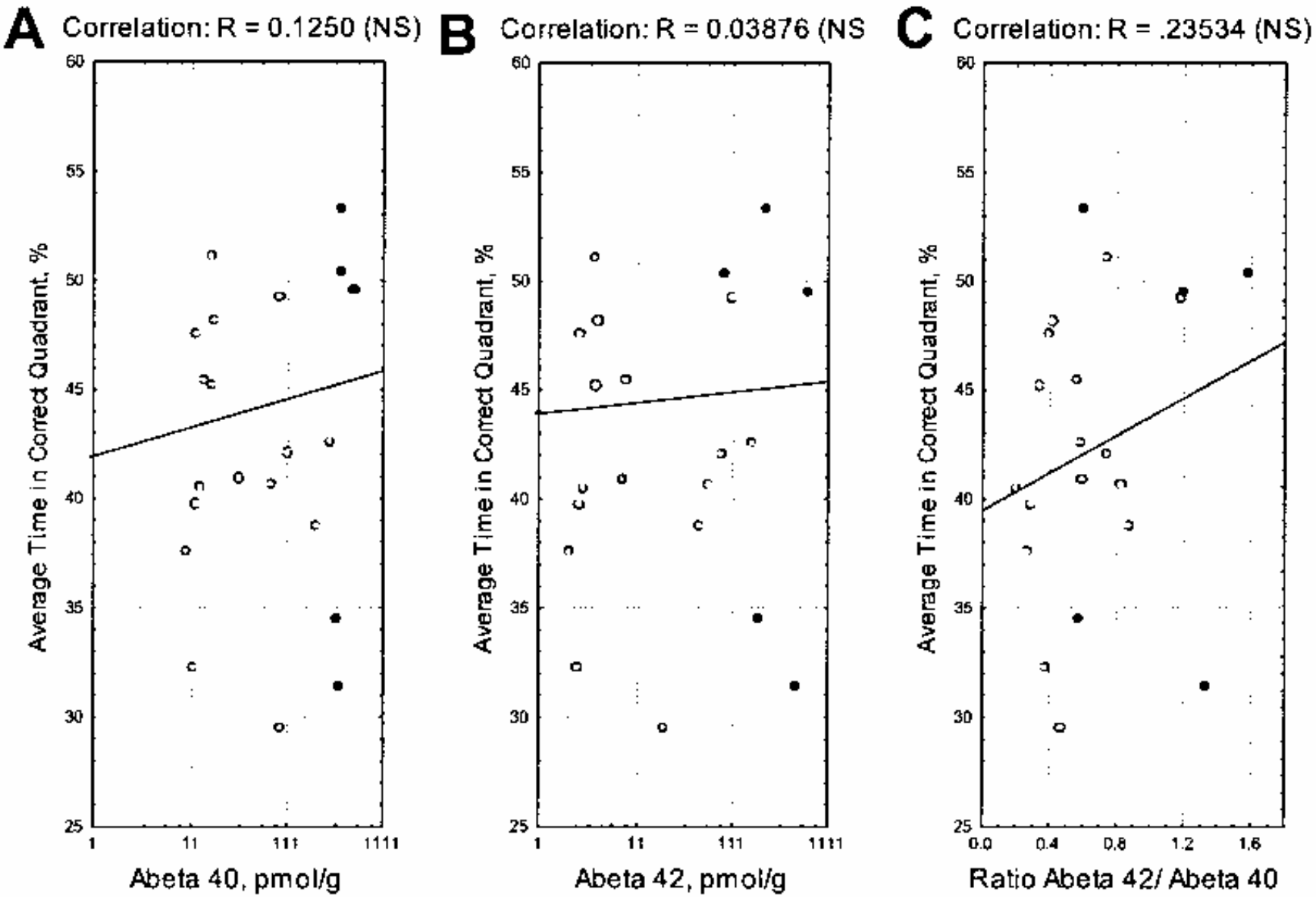
APPswe Mice: Exponential Increase of Aβ in the Brain



Normal Cognition in Two Independent Lines of Mice Expressing APPswe



No Correlation Between Aβ40 or 42 Levels and Cognition in APPswe Mice



APPswe Mice: No Cognitive or Motor Deficits (7 – 24 Months)

	Longitudinal study (noncongenic mice)					***Crossectional*** (congenic)		
Tasks	7 mo	12 mo	13 mo	18 mo	24 mo	7 mo	12 mo	24 mo
Body Weight	NS	NS	NS	NS	NS	Tg >NTg	NS	NS
SensoriMotor Tasks	NS	NS	------	NS	Z score Tg <NTg	NS	Z score Tg <NTg	NS
Open Field	NS	NS	------	NS	NS	NS	NS	NS
Plus Maze	NS	NS	------	NS	NS	NS	NS	NS
Visual Discrimination	NS	NS	------	NS	NS	NS	NS	NS
Straight Swim	NS	NS	------	NS	NS	NS	NS	NS
Place Discrimination	NS	NS	NS	NS	NS	NS CorQ Tg <NTg	NS	NS
Spontaneous Altern.	NS	NS	------	NS	NS	NS	NS	NS
Radial Maze	NS	------	------	------	NS	------	------	------
Inhibitory Avoidance	------	------	------	------	------	NS	NS	NS
Active Avoidance	----	------	------	------	------	Tg better NTg in Avoid (sess 1,3)	NS	NS

What have others found?

Many publications can be found which report that expression of mutant APP at levels that are either sufficient, or insufficient, to induce amyloid deposition can impair cognition in mice prior to the appearance of amyloid pathology.

In some cases, the levels of expression required to induce phenotypes are at or below the level of detection (especially true in mice that express the last 100 amino acids of APP as a truncation fragment).

APPswe / *PS1dE9* Mice

APPswe: chimeric Mo/Hu *APPswe*
strain background [C3H/HeJ x C57BL/6J]
10^{th}-11^{th} generation of backcrosses to C57BL/6J

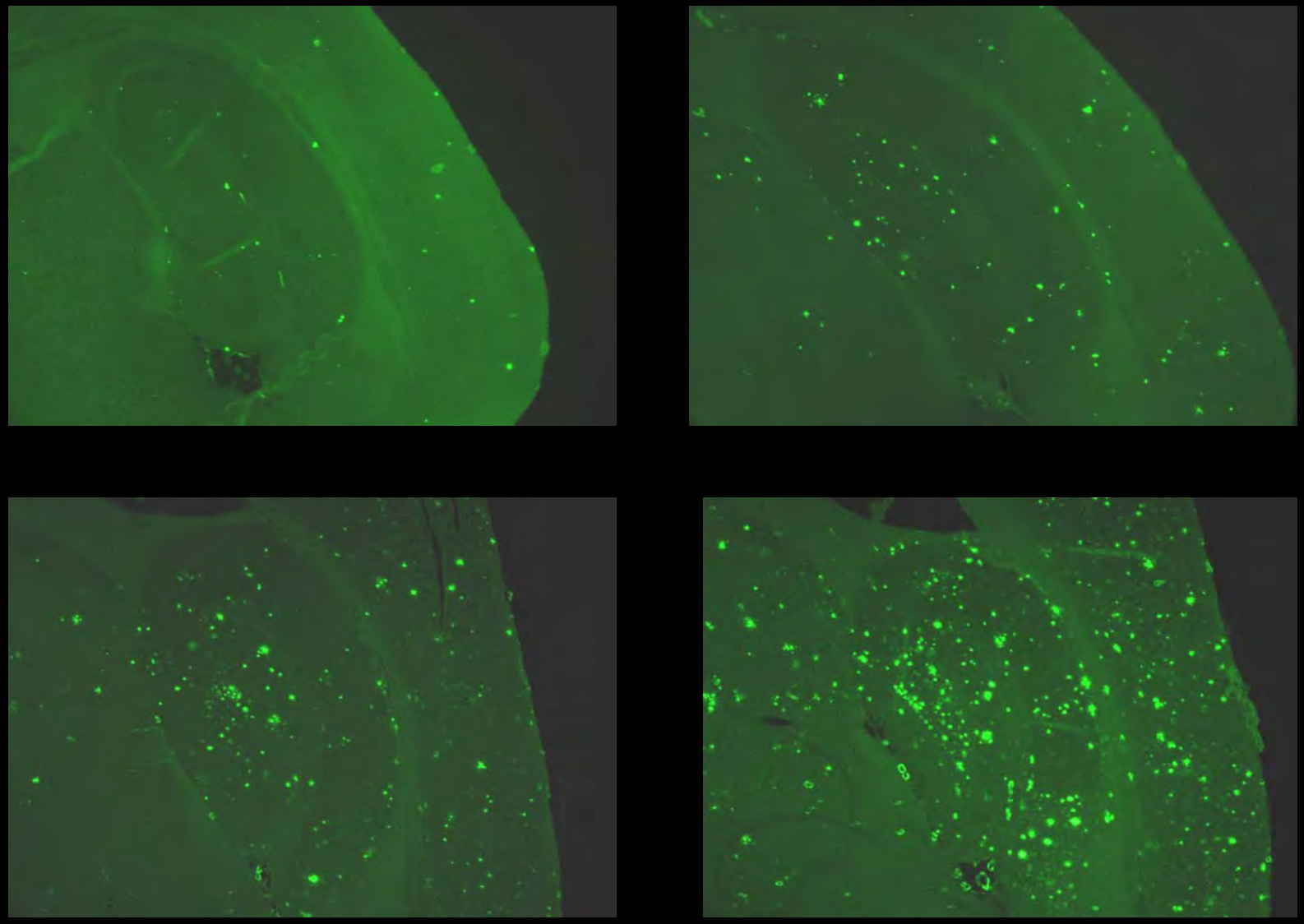
PS1dE9: strain background [C3H/HeJ x C57BL/6J]
6^{th} generation of backcrosses to C57BL/6J mice

APPswe /PS1dE9 Mice

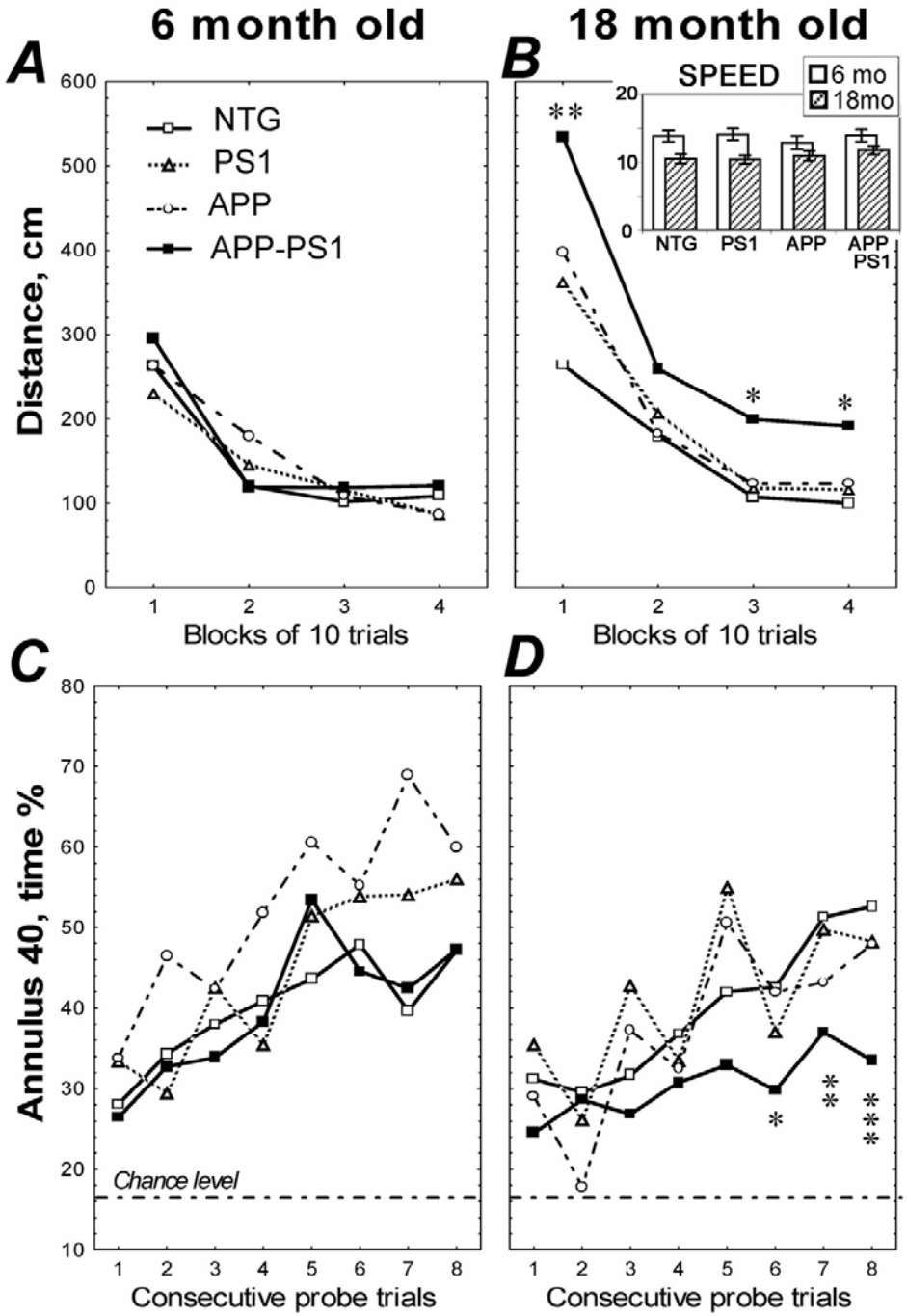
Plaques Appear at 5-6 months of age

6-mo old males

18-mo old males

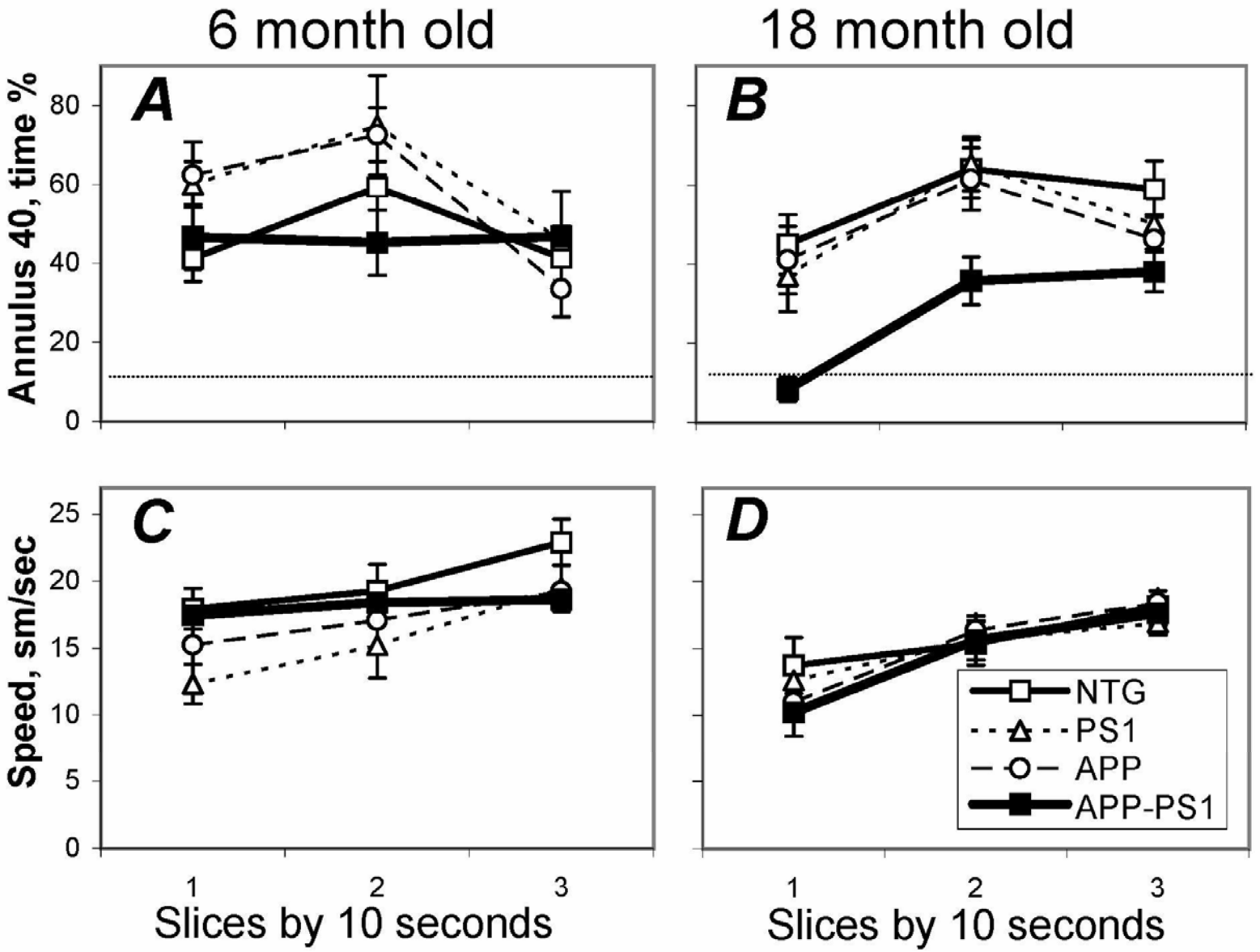


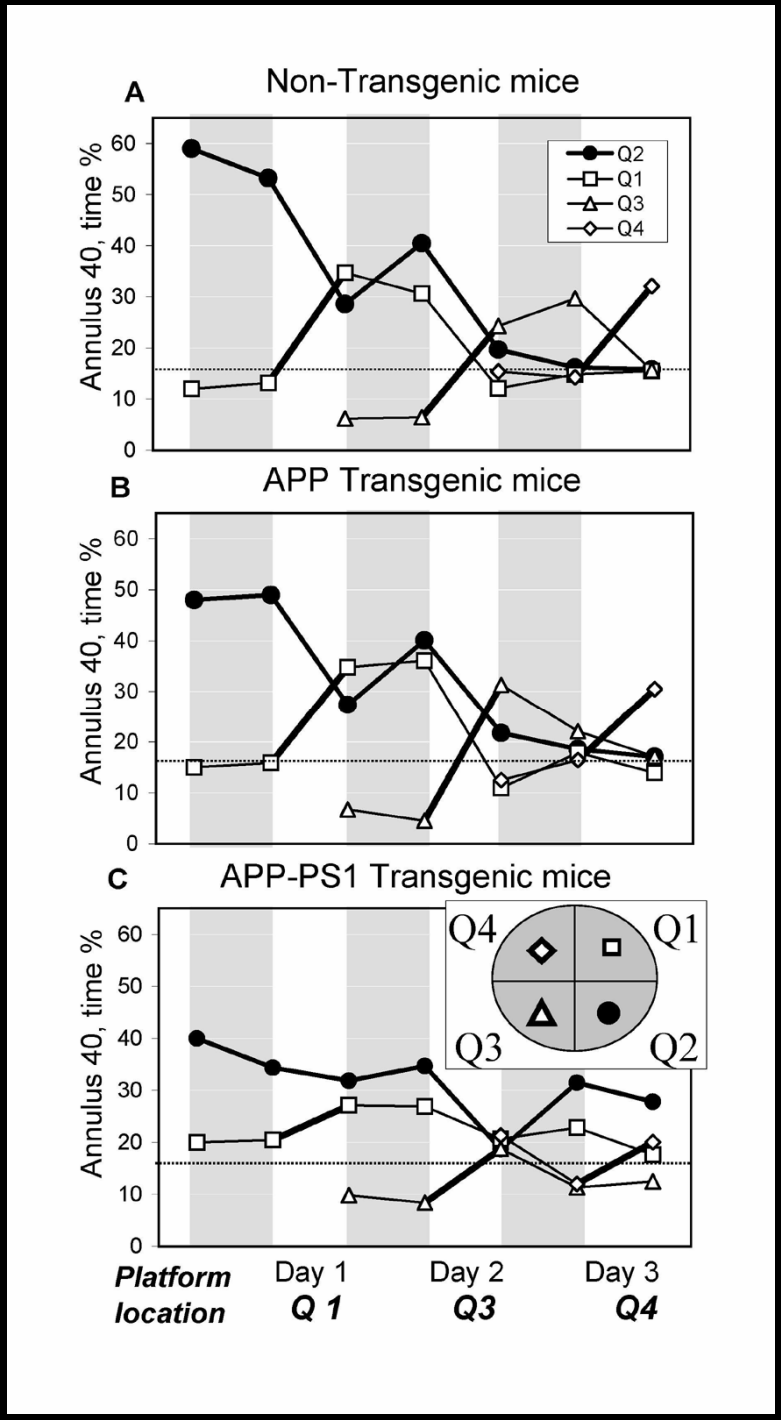
ThioflavinS



APPswe/ *PS1dE9* Mice: Place Discrimination in Water Maze

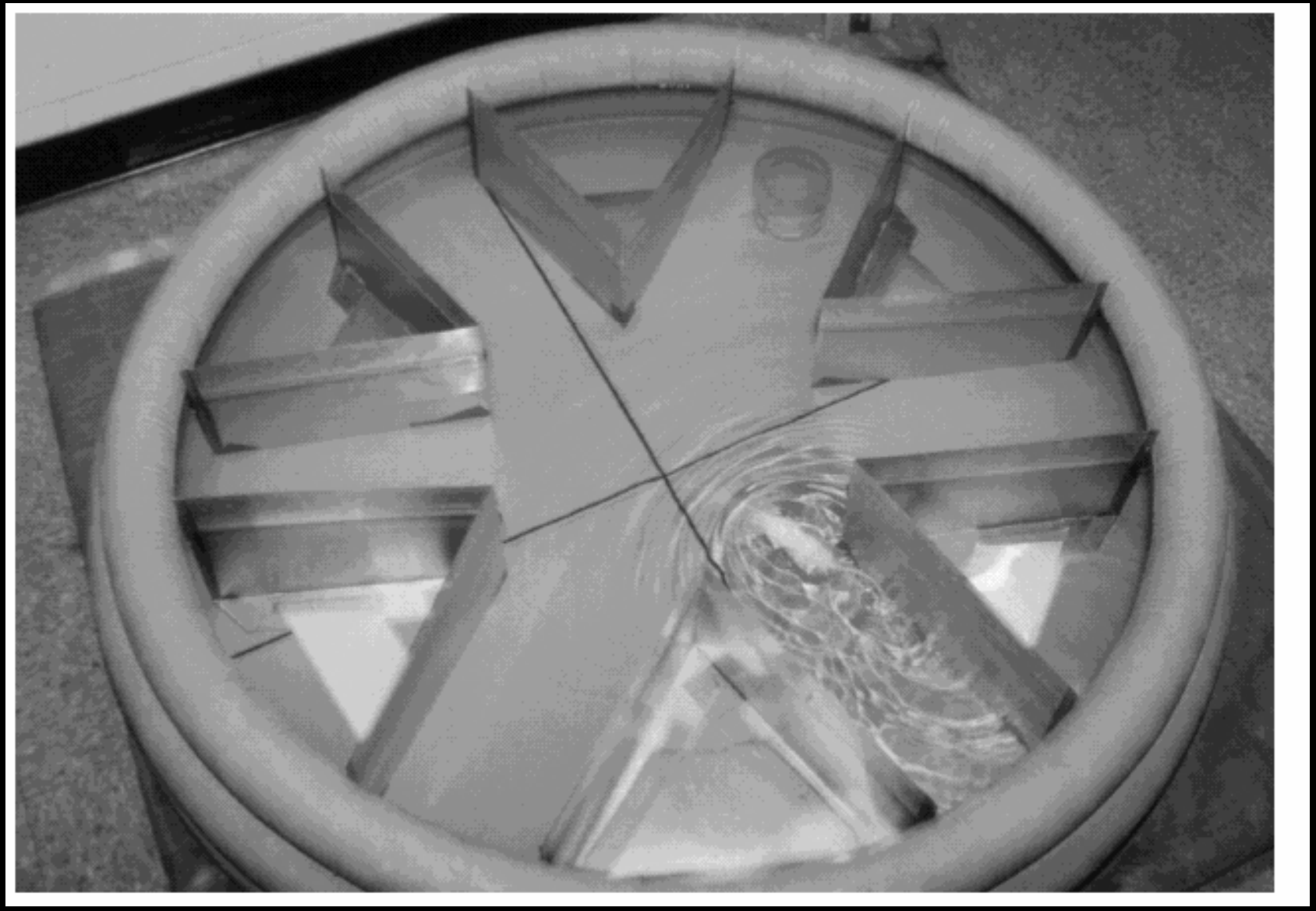
Probe trial deficits are greatest in the first 10 seconds of the trial





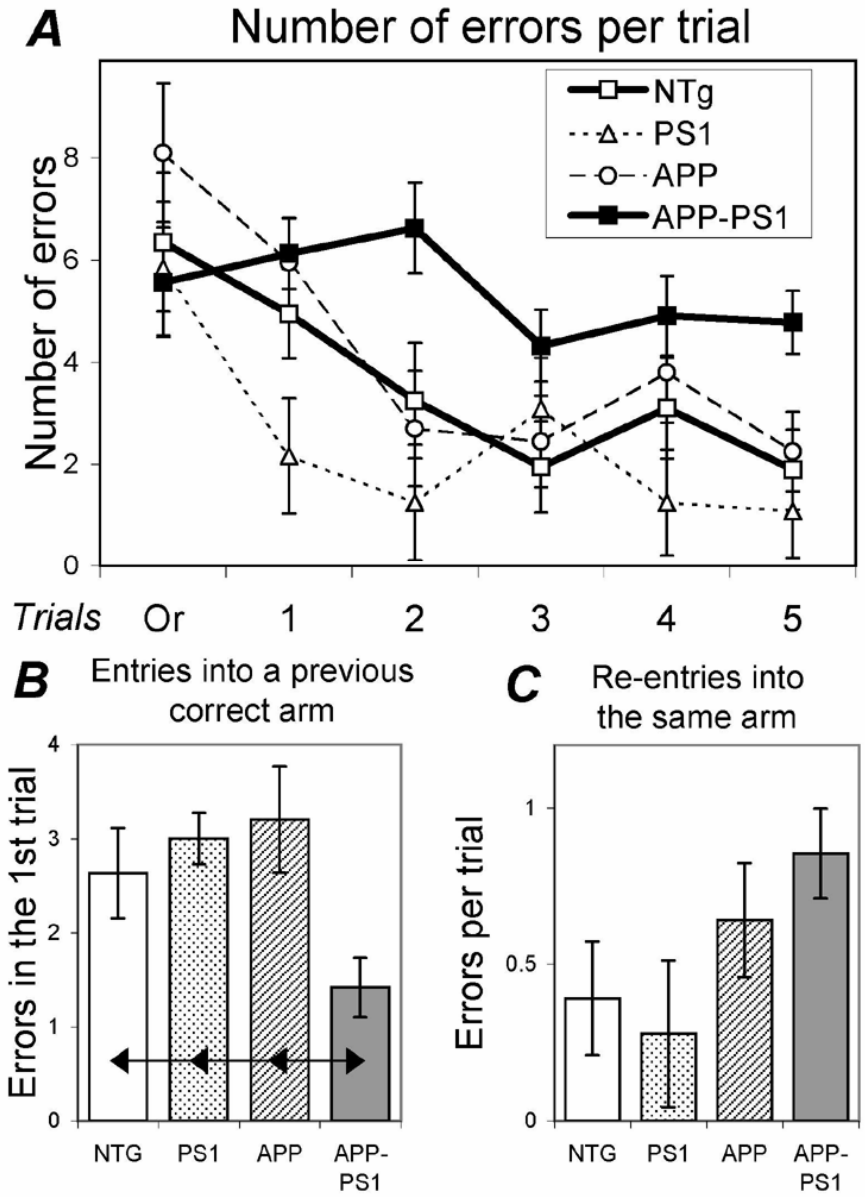
Repeated Acquisition Task Reveals Deficits in Working Memory With Much Milder Deficits in Reference Memory in 18-month-old APPswe/PS1dE9 mice

APPswe + *PS1dE9* Mice: 6-Arms Radial Water Maze

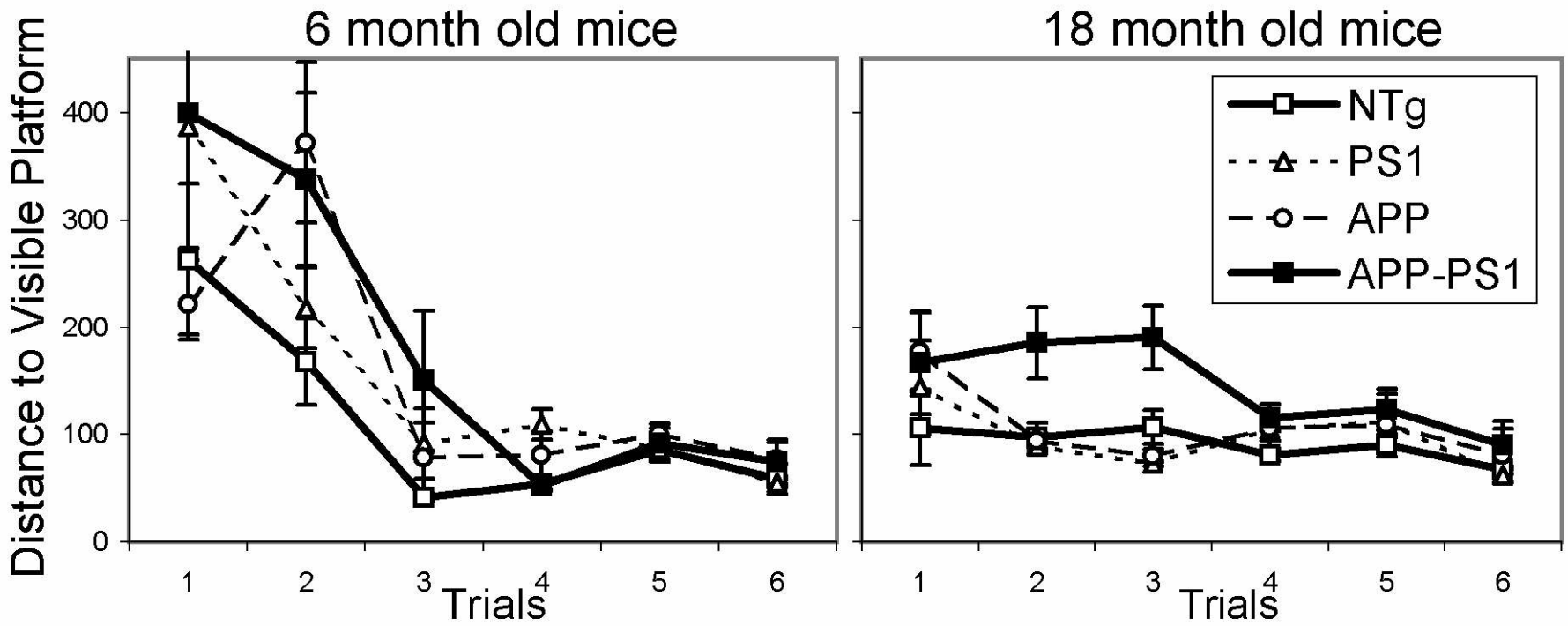


Picture from G .W. Arendash *et al* . / *Brain Research* 891 (2001) 42– 53

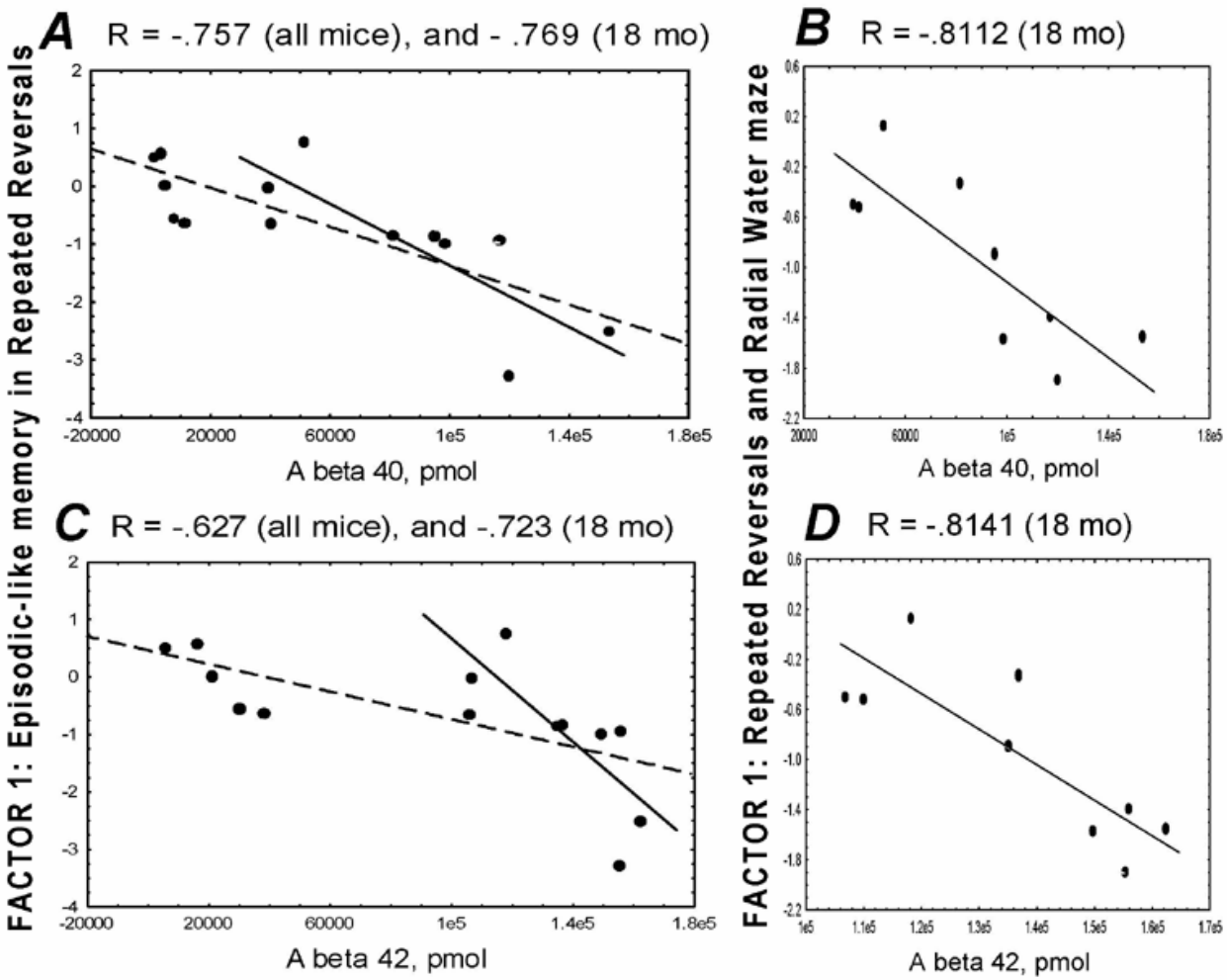
APPswe/PS1dE9 Mice (18-mo males): Deficits in 6-Arm Radial Water Maze



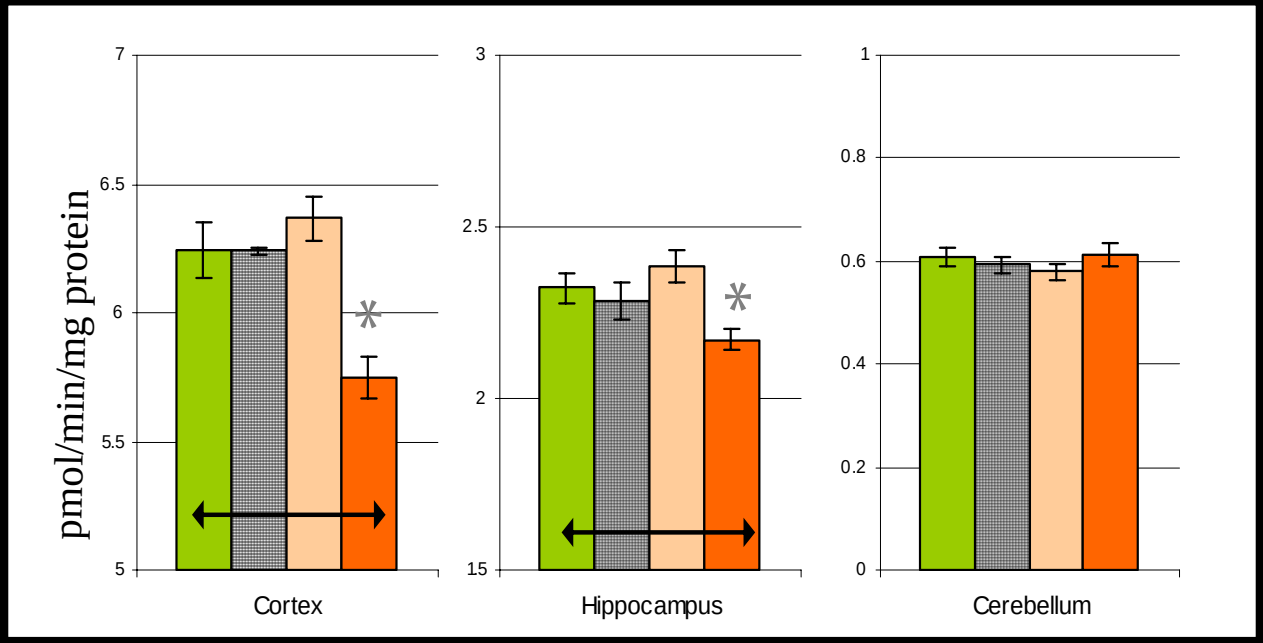
Vision in Normal in APPswe/PS1dE9 Mice



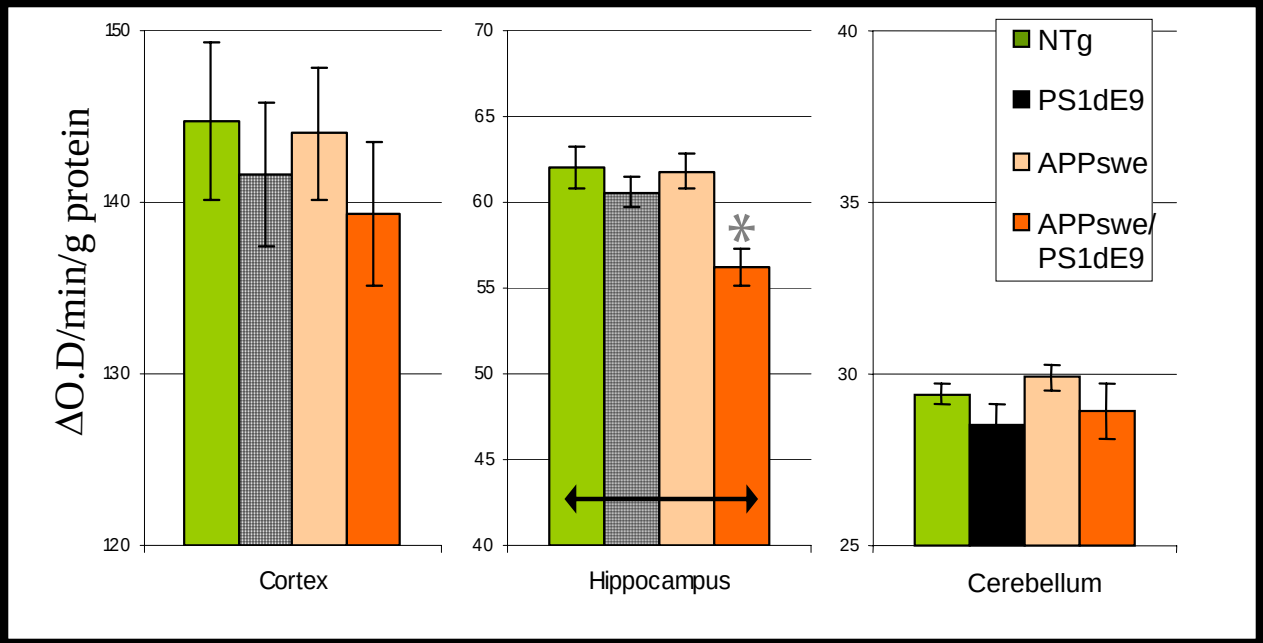
Memory Deficits Correlate With High Aβ Burden



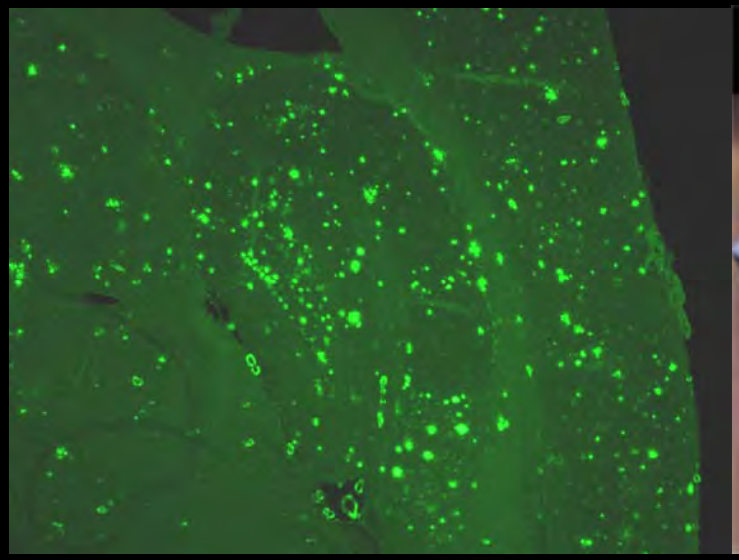
Reduced ChAT activity in Cortex and Hippocampus

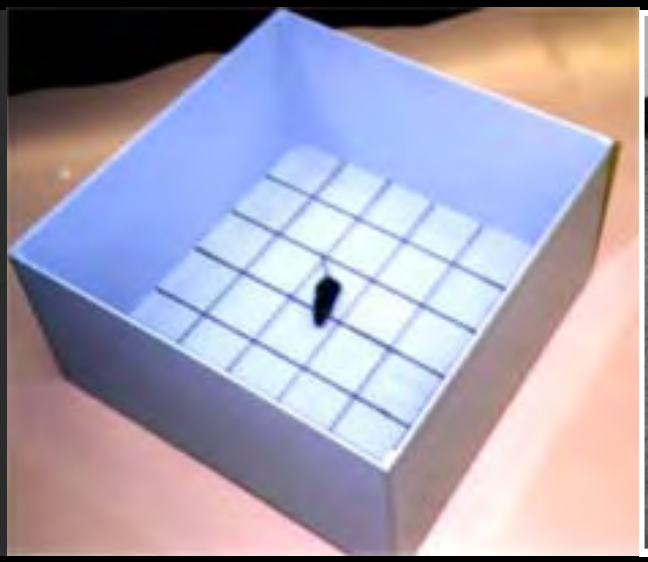


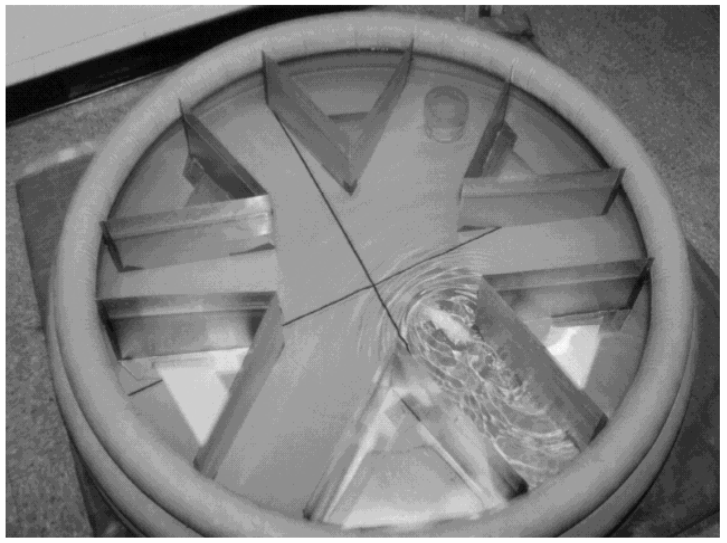
Reduced AChE activity in Hippocampus



Clinical-Pathological Correlations in APPswe/PS1dE9 Mice







- Amyloid plaques form prior to onset of cognitive impairments

- Amyloid burdens are relatively high in cognitively impaired mice.

- Behavioral deficits correlate with mild diminutions of cholinergic markers

What have others found in the most used models?

Tg2576 model – not congenic: Behavioral deficits most robust after the appearance of amyloid pathology. However, the correlation between amyloid load and performance is reported to be poor. Interpretation: amyloid is marker for other species of Aβ that are the most toxic (oligomers also called ADDL's).

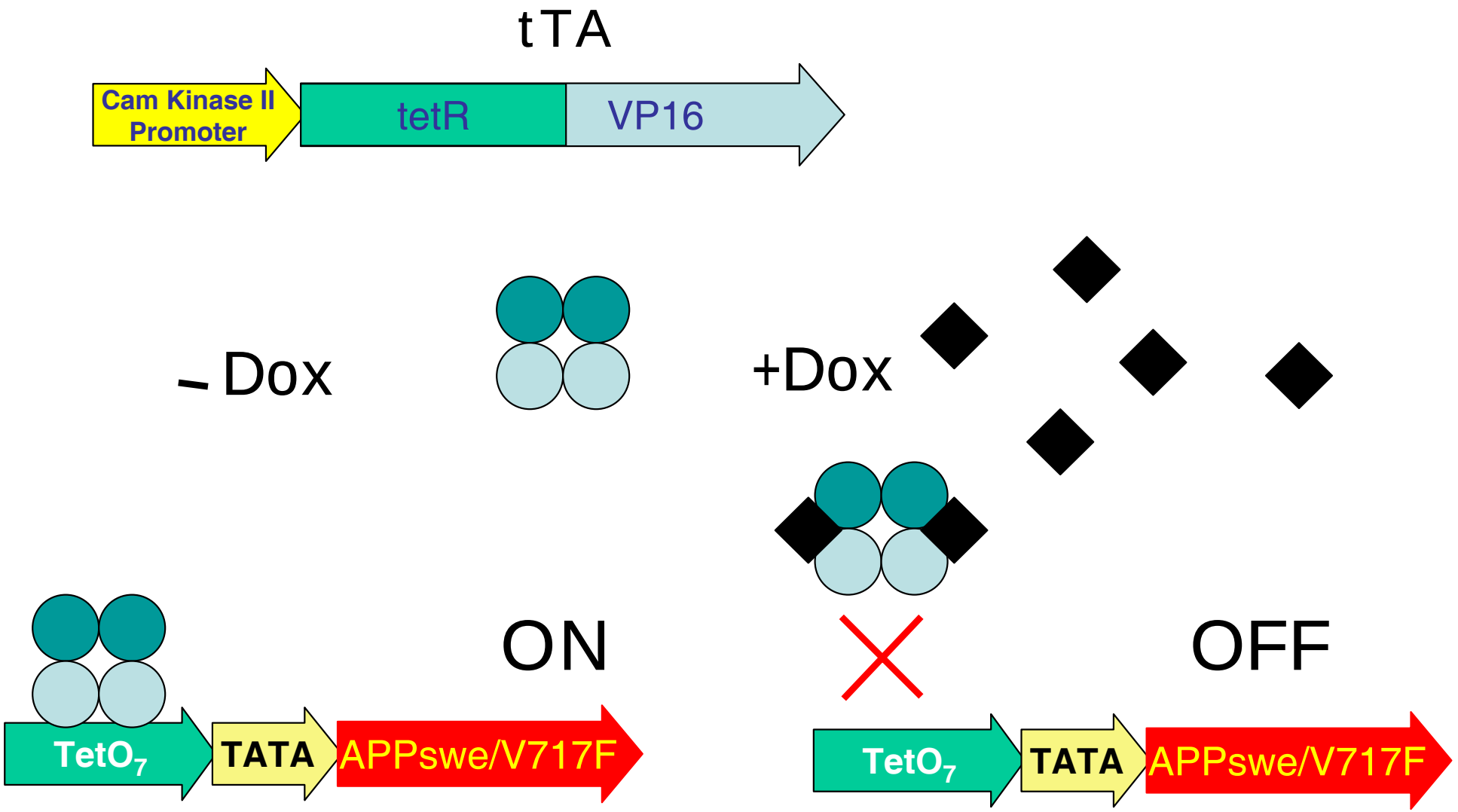
PDGF model – congenic: Robust behavioral deficits apparent after amyloid onset. However, not consistent among different lines.

Thy-1 model – congenic: Robust behavioral deficits apparent after amyloid onset.

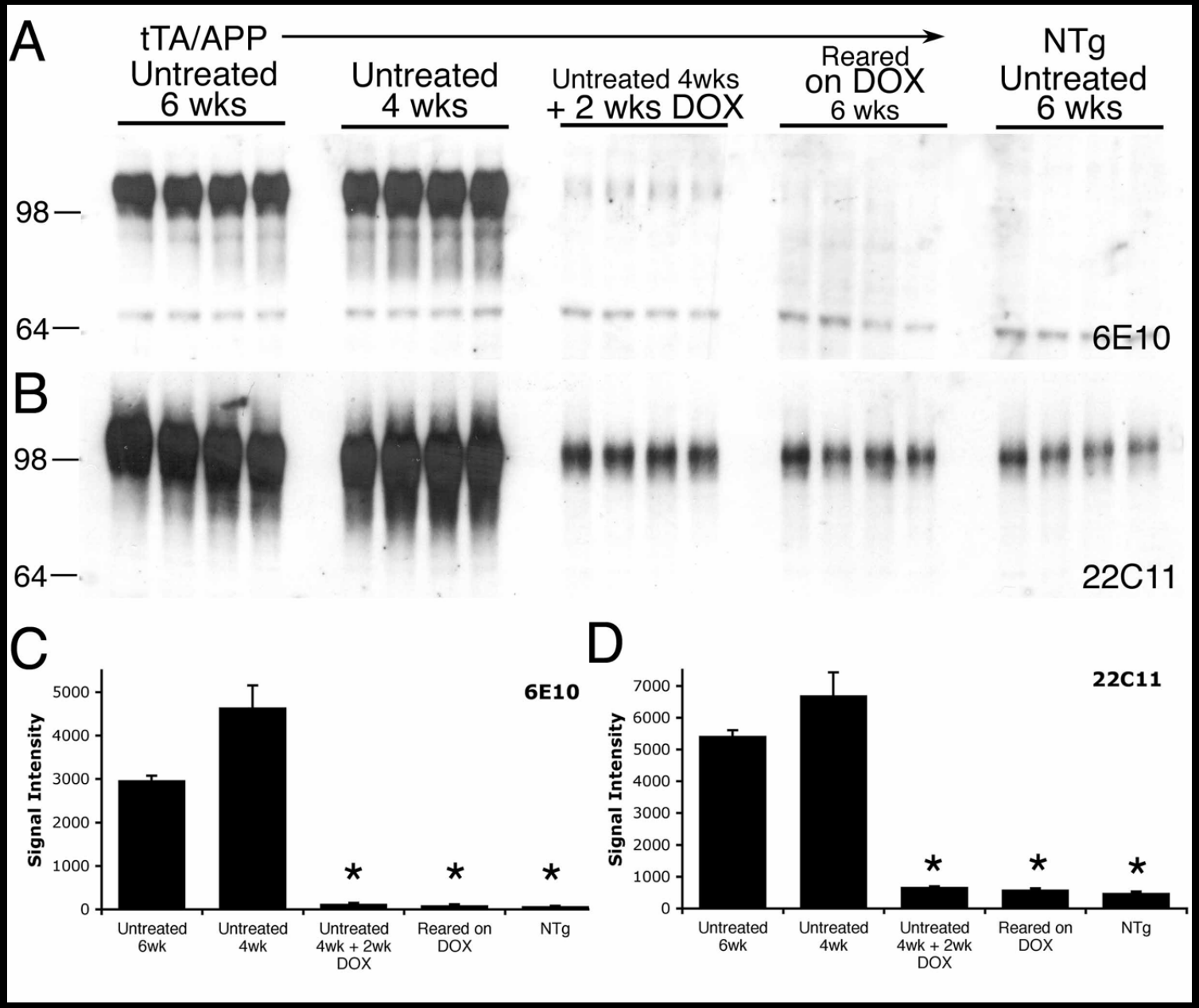
By all accounts, amyloid would seem to be a very good therapeutic target for AD.

If we build a genetic mimic of therapy targeting amyloid, how well does the CNS recover?

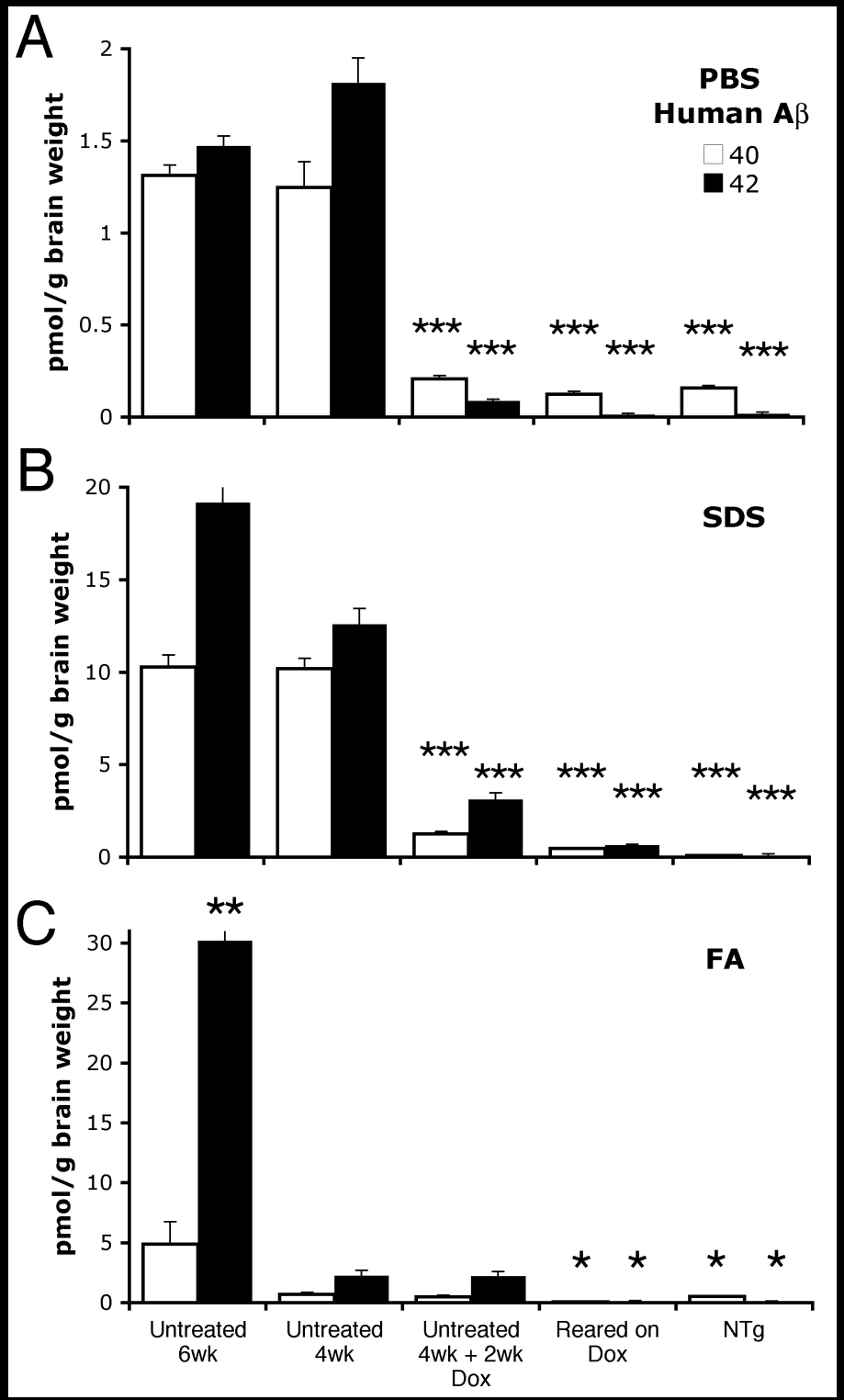
Molecular Mechanics of Tet-Off

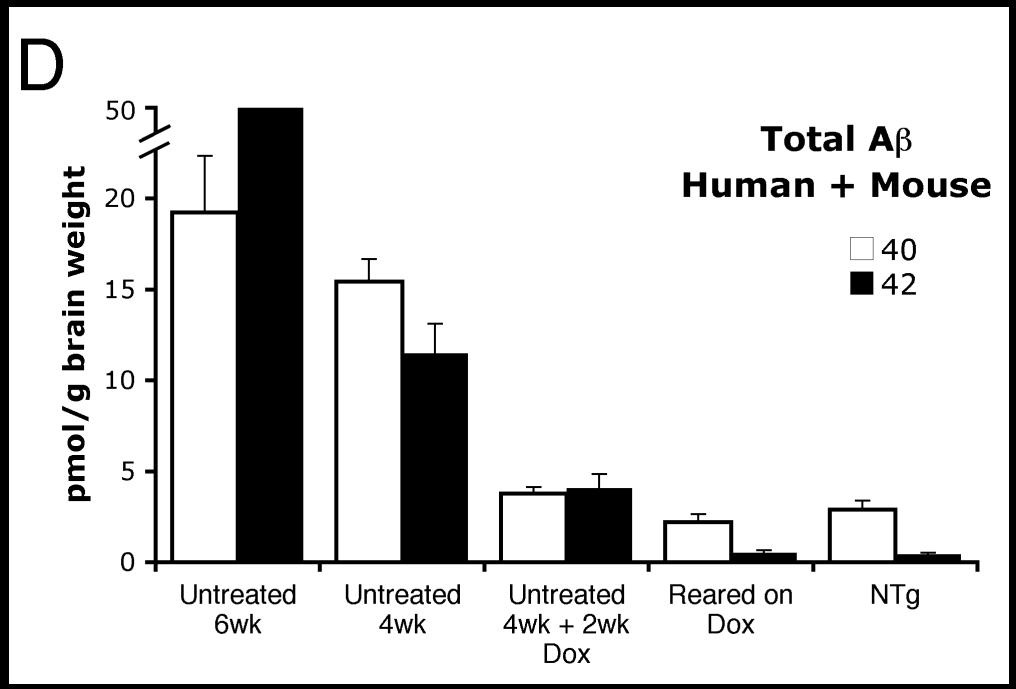


Doxycycline rapidly diminishes expression of mutant APPswe/ind

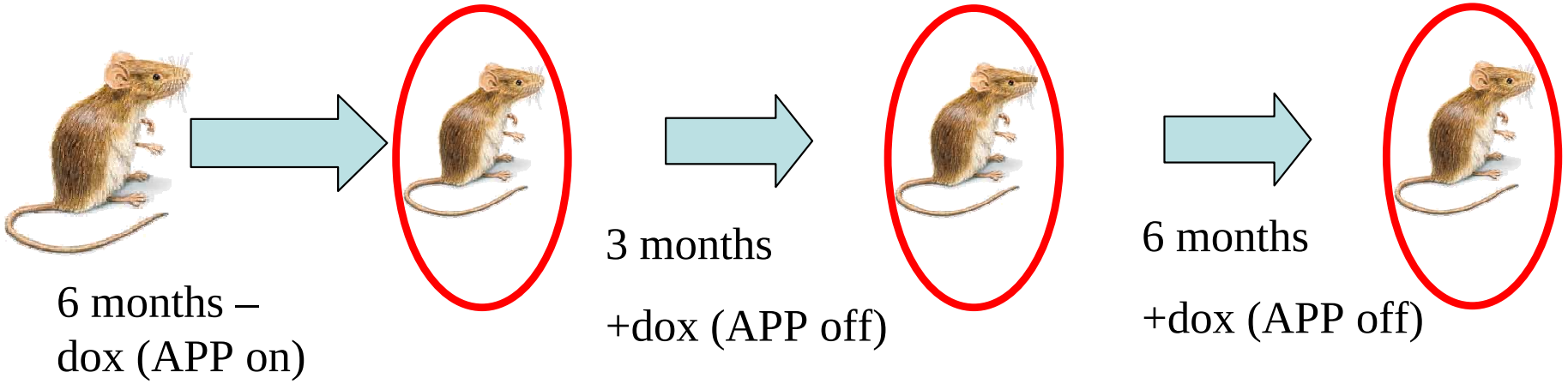


Reductions in mutant APP expression are paralleled by rapid reductions in the production of A-beta peptide





Regulated Expression of mutant APP (APPswe/ind)
Joanna Jankowsky



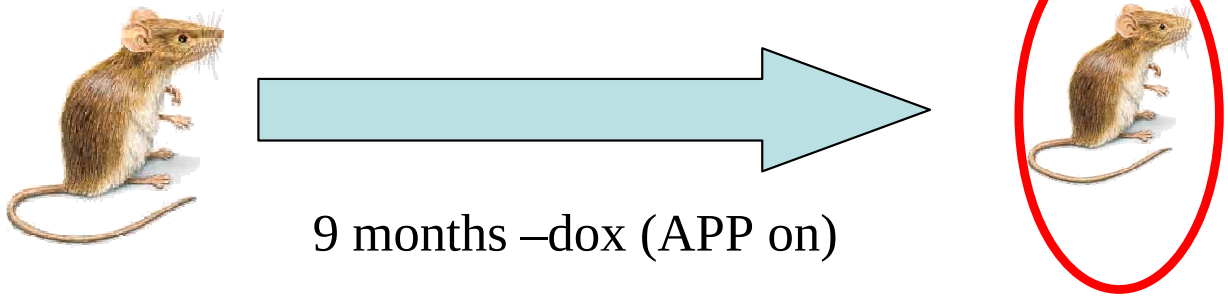
6 months –
dox (APP on)

3 months

+dox (APP off)

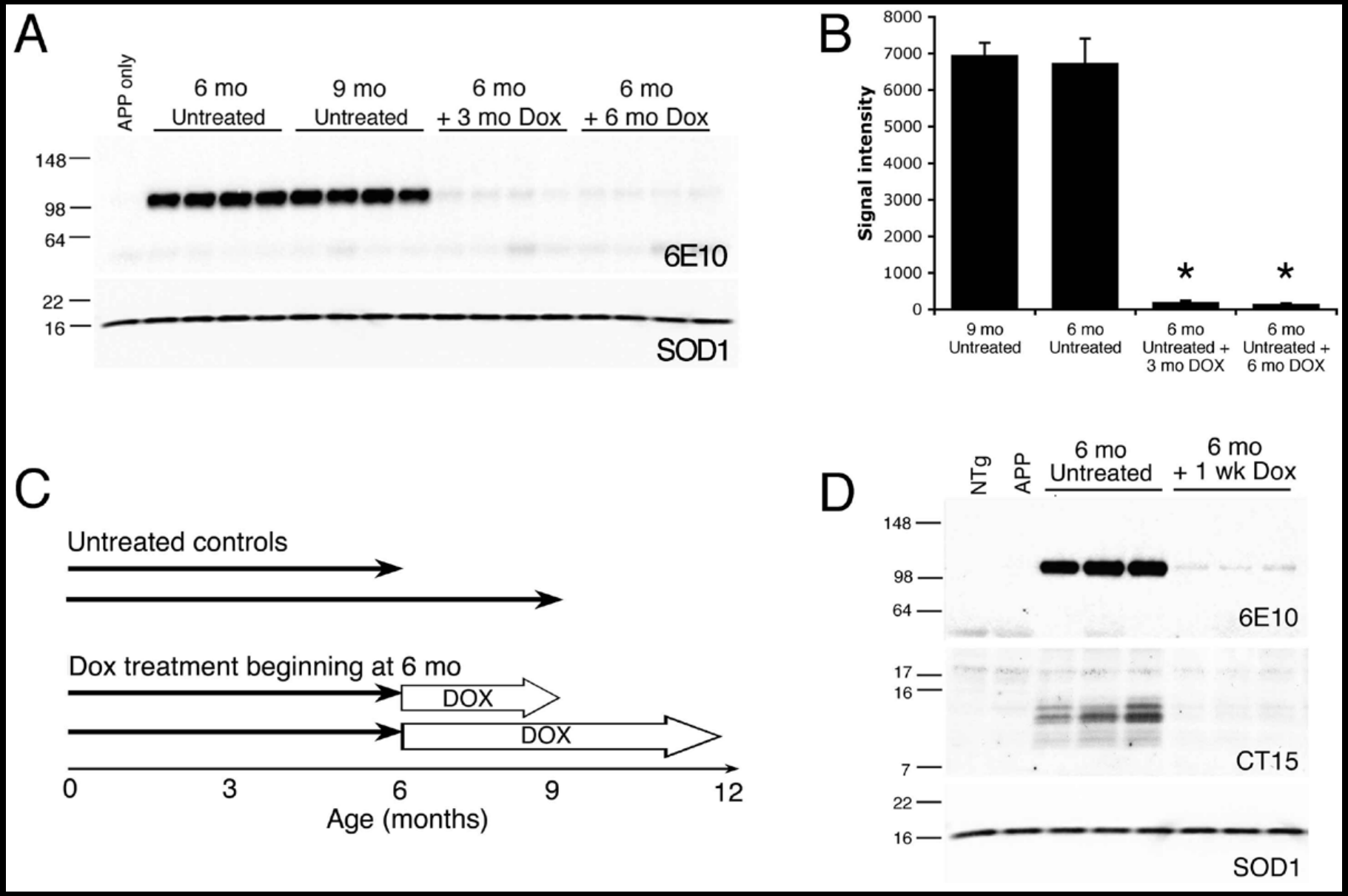
6 months

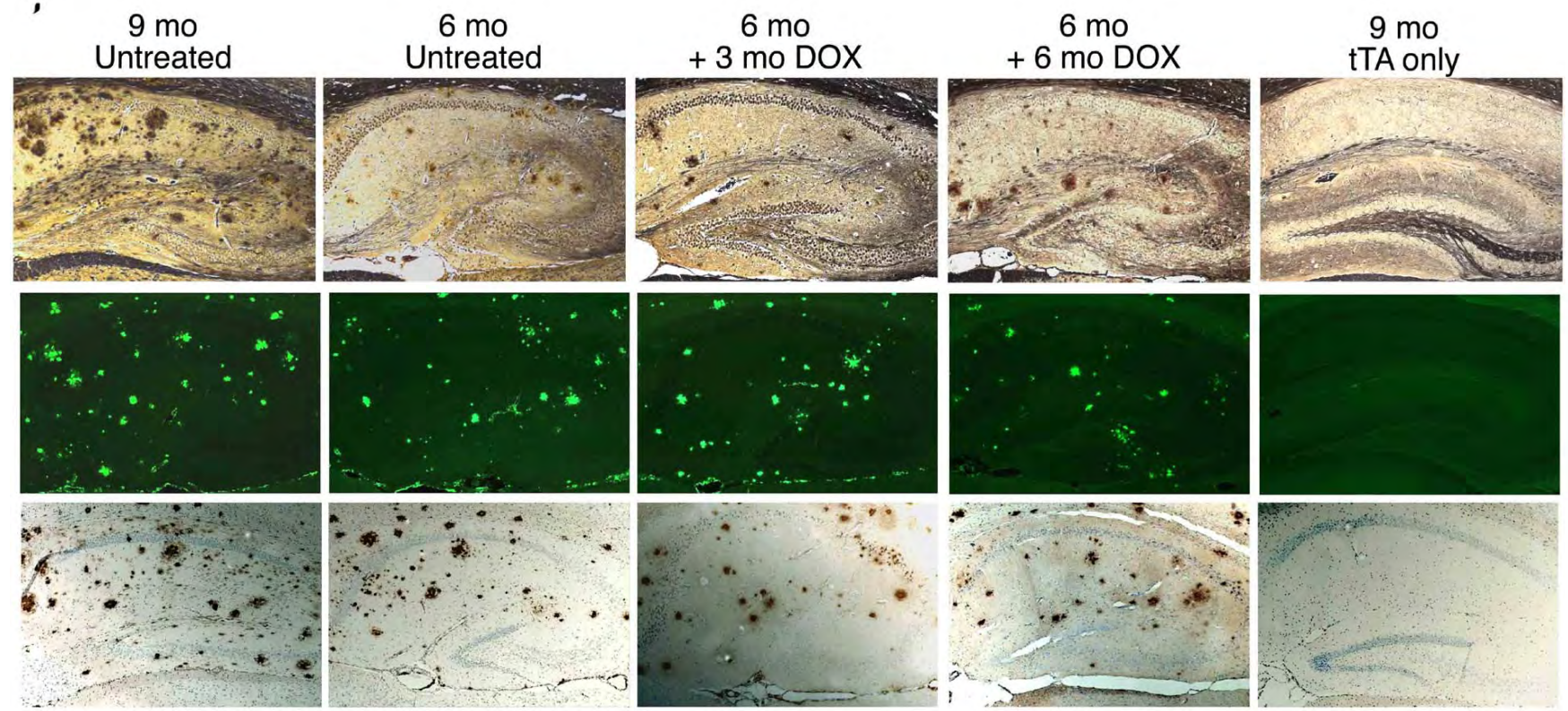
+dox (APP off)

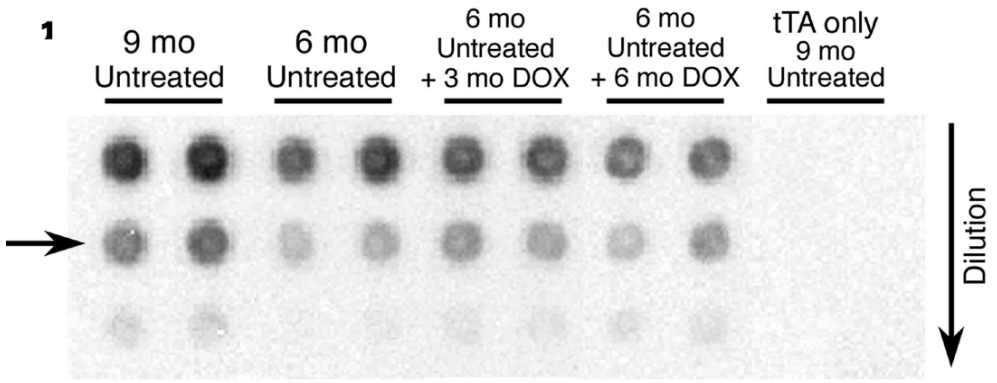


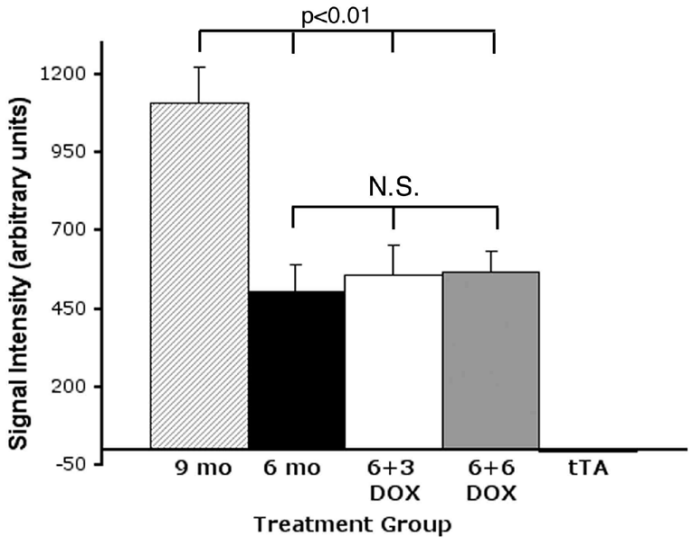
9 months –dox (APP on)

Sustained suppression of APP expression

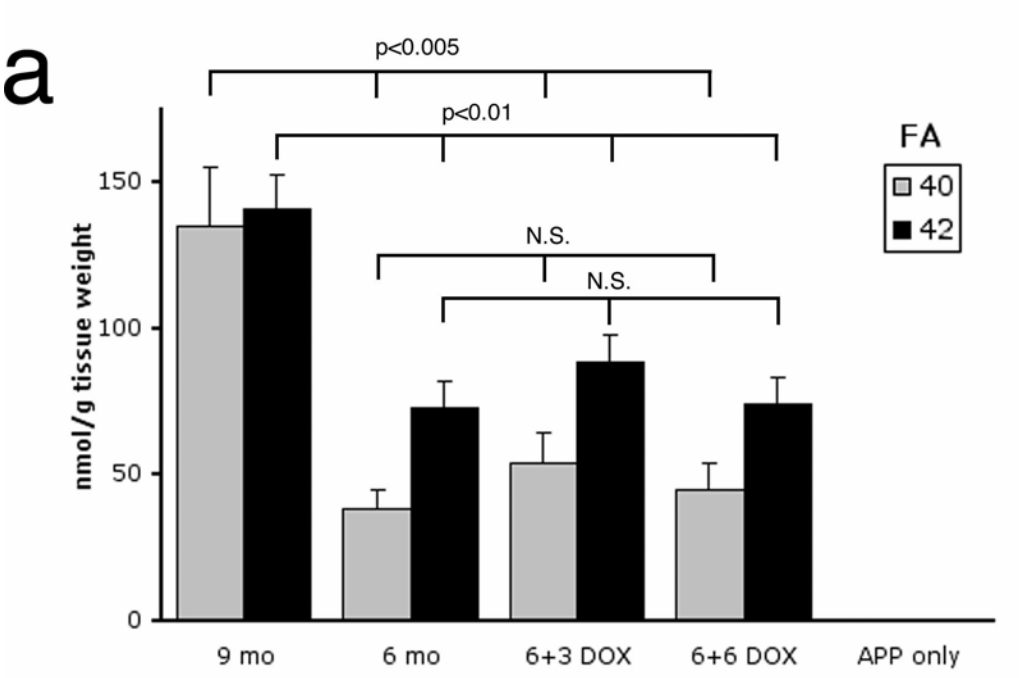


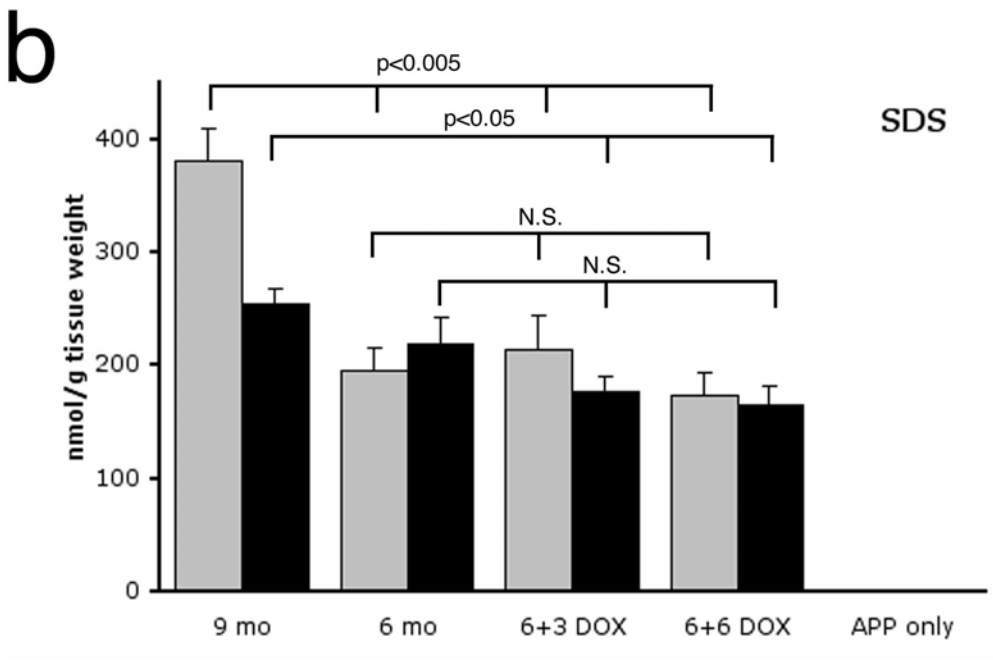

9 mo
Untreated
6 mo
Untreated
6 mo
+ 3 mo DOX
6 mo
+ 6 mo DOX
9 mo
tTA only

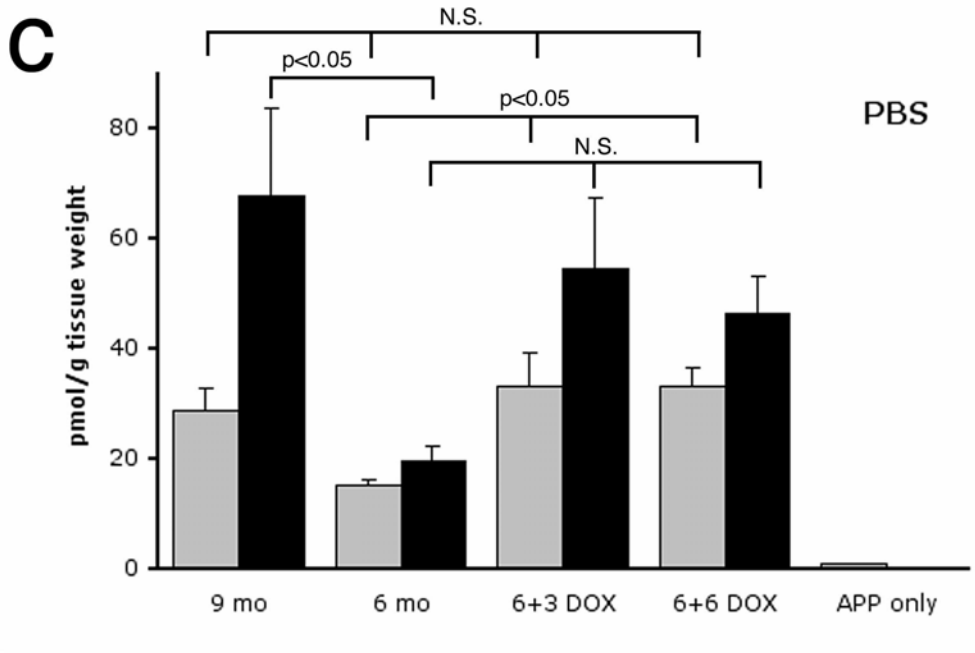

9 mo
Untreated
6 mo
Untreated
6 mo
Untreated
+ 3 mo DOX
6 mo
Untreated
+ 6 mo DOX
tTA only
9 mo
Untreated
Dilution


p<0.01
N.S.
1200
950
700
450
200
-50
Signal Intensity (arbitrary units)
9 mo
6 mo
6+3
DOX
6+6
DOX
tTA
Treatment Group

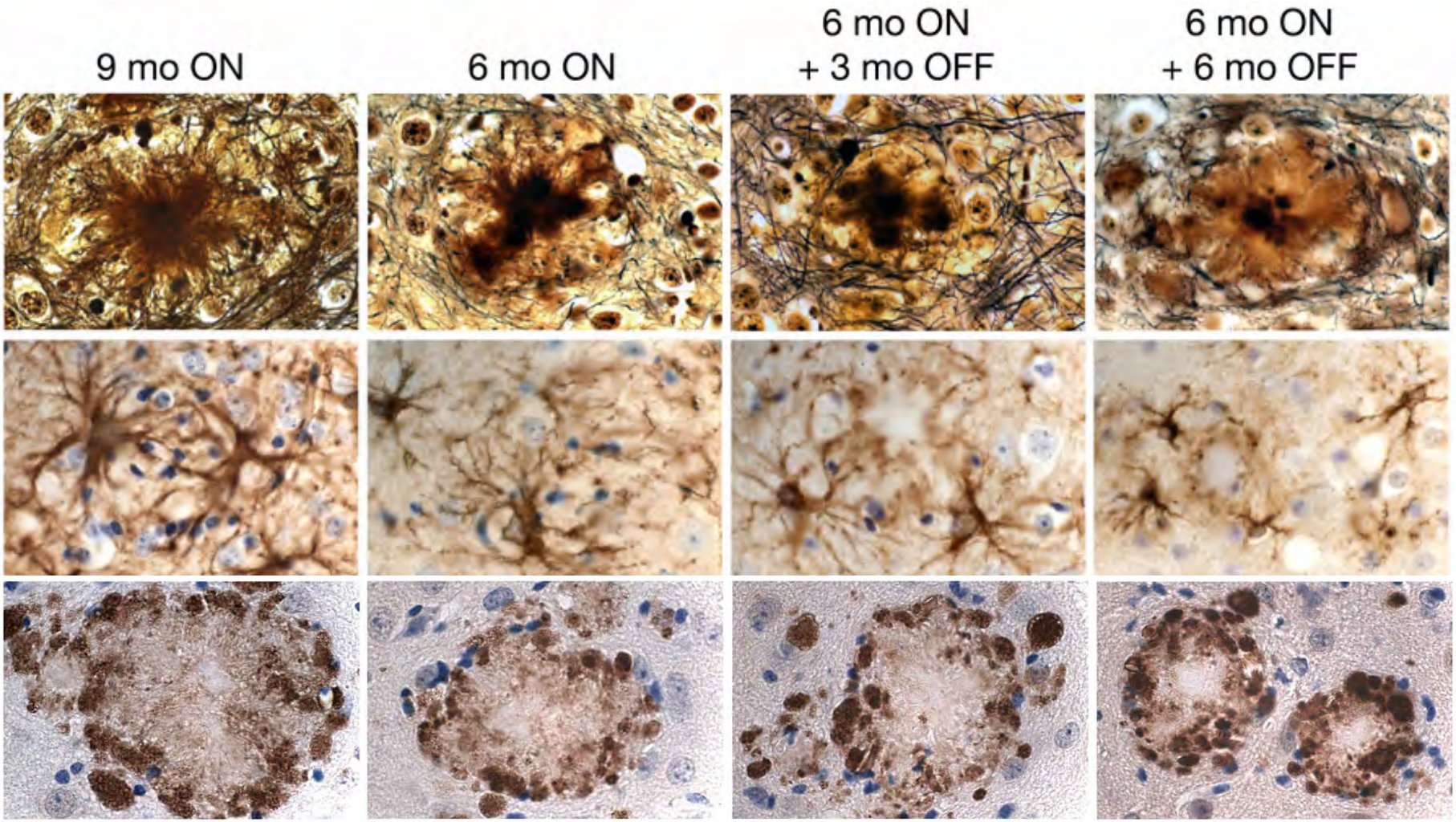
Persistent levels of insoluble A-beta in animals chronically exposed to doxycycline.







Persistent neuritic abnormalities and astrocytic responses and ubiquitin immunoreactive neurites



Result Summary

- As one would expect, eliminating Aβ production halts the progression of pathology.
- Somewhat surprisingly, removal of mature amyloid deposits requires an interval of time greater than that required to create these lesions.
- The estimated differential between A-beta inputs pre- and post-treatment with doxycycline is about 30 fold: Clearly equilibrium vastly favors the stability of amyloid deposits in vivo.
- The clear take home message is that for therapeutics targeted to the production of A-beta, early intervention may be essential if not absolutely required.

What have others found

Thy-1 mutant APP model – congenic: Lentiviral delivery of BACE RNAi lowers amyloid burden at site of injection by >2-fold within 4 weeks.

Tet-off Tau model – noncongenic: Tau pathology does not clear after suppression of transgene expression. However, improvements in behavior noted.

Antibody-mediated-clearance: Injections of anti-Ab antibodies directly into hippocampus induces rapid clearance of amyloid plaques. In APP/PS1/Tau model – tau amyloid/Aβ pathology and Tau pathology rapidly clear.

Where do we go from here?

Determine whether doxycycline has an effect on amyloid metabolism.

Examine cognitive performance of mice with inhibited expression of mutant APP (with and without amyloid).

Develop the model as a system to identify stimuli that accelerate the clearance of amyloid.

Production of Transgenic Mice
Nancy Jenkins & Neal Copeland (NCI)

Measurements of Aβ – Linda and Steve Younkin, Mayo Clinic Jacksonville

Aβ and Tau Aggregates

Guilian Xu

Mouse Psychologist

Alena Savonenko

APP Transgenic Mice
Environmental Enrichment

Joanna Jankowsky

Hilda Slunt

Victoria Gonzales

Michael Coonfield

David Fromholt

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